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Relating Values of Selected Benthic Macroinvertebrate Metrics from D-net to Surber Sampler in Two Southeast Wyoming Streams

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ABSTRACT

Samples were taken from two southeast Wyoming streams to determine if values of selected benthic macroinvertebrate metrics derived from using a D-net could be related to values derived from using a Surber sampler. Conditions under which samples were collected were variable, ranging from severely polluted to non-polluted over two seasons. Metrics evaluated were the Shannon-Weiner index and the random runs value plus four measures of richness; Ephemeroptera taxa richness, Ephemeroptera/Plecoptera/Trichoptera taxa richness, total taxa richness, and Plecoptera taxa richness. Regression analysis indicated that linear relations existed between average metric values estimated through use of the D-net and Surber sampler. These results indicated that average D-net values for these six metrics can be expressed as average Surber sampler values, and vice versa, in these two streams when using either the D-net or Surber sampler in the manner described. This study presented two ways (i.e., the *F* test or the model validation technique) to test for transportability of functions (i.e., regression models), which were used to convert the results from one sampling method to results from another, and vice versa.

KEY WORDS: Benthic insect communities, metrics, Surber sampler, D-net, metric conversion

INTRODUCTION

Many sampling devices have been utilized and described to assess benthic macroinvertebrate assemblages (see reviews in Hynes 1970; Elliott and Tullett 1978; Resh 1979; Peckarsky 1984; Hellawell 1986; Resh and Jackson 1993). Of these, several have been used to collect benthic macroinvertebrates for assessing anthropogenic disturbances to streams and rivers (Chutter 1972; Whitehurst 1991; Dieter et al. 1996). In general, sampling methods are chosen based on the objectives of projects and physical characteristics of the sampling sites.

Unfortunately, there is little standardization regarding which sampling device to use or how a sampling device is to be used (Carter and Resh 2001). Such lack of standardization can limit the extent to which benthic macroinvertebrate data can be shared among governmental agencies and private entities. As a precaution, the sharing of data should be done after some form of comparison is made to ensure that values from one data set can be expressed as values of another.

A few studies have been conducted in which captured benthic macroinvertebrates were compared between sampling methods (lentic systems: Garcia-Criado and Trigal 2005; Jurado et al. 2008; lotic systems: Storey et al. 1991; Barton and Metcalfesmith 1992; Blocksom and Flotemersch 2005; Friberg et al. 2006). In these studies, different sampling methods were evaluated using multiple macroinvertebrate indices. We could find no studies, however, that explored how values of specific benthic macroinvertebrate metrics,

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derived from using one sampling device in a given way, related to values that were derived from using that same sampling device but in a different way or to values derived from using a different sampling device altogether.

One commonly used sampling device for assessing anthropogenic influences on lotic systems is the Surber sampler (Surber 1937; Winterbourn 1985; Resh and McElravy 1993). This device is used in quantitative studies of benthic macroinvertebrates because when it is placed on the benthos it covers a fixed, quantifiable area. When using the Surber sampler, 3–5 samples (replicates) are typically collected from a site (Resh and McElvray 1993). Collecting and processing benthic macroinvertebrates captured in 3–5 Surber samples generally takes more time than collecting and processing benthic macroinvertebrates captured in D-nets (Storey et al. 1991). The Surber sampler is the sampling instrument specified in the official biomonitoring protocols for the biological monitoring of lotic systems in some U.S. states, such as Montana and Wyoming.

The D-net is a 'convenient qualitative method [for assessing benthic macroinvertebrate communities] that does not rely on cumbersome or expensive equipment' (Mackey et al. 1984). The D-net is typically regarded as a semi-quantitative sampling method because it does not cover a fixed area of streambed. Rather, it is used in a semi-quantitative fashion by sampling for a fixed amount of time. Traditionally, and typically, either a single D-net sample or a composite of several D-net samples is used to represent the benthic macroinvertebrate assemblage at a site. The D-net is the most commonly specified sampling device in rapid assessment protocols of most state agencies (Carter and Resh 2001). These were designed to both minimize effort when assessing aquatic ecosystems and give results that are easily interpreted and understood by nonspecialists (Resh and Jackson 1993).

As the Surber sampler and D-net are perhaps the most commonly used devices for sampling benthic macroinvertebrates in lotic systems, we wondered if the information derived from one could somehow be related to the other, either straight away or through a mathematical function. This is of interest because converting benthic macroinvertebrate data, obtained through use of different sampling methodologies, would reduce duplicated effort and increase the availability of data. We therefore conducted a study to determine if some benthic macroinvertebrate data when collected through use of a Surber sampler could be expressed as benthic macroinvertebrate data when collected through use of a D-net. Benthic macroinvertebrate data used in this study were collected under variable environmental conditions, including polluted (mine-impacted) and non-polluted lotic systems over two seasons (summer and autumn).

MATERIALS AND METHODS

Study Area and Sample Site Selection

The mine-impacted and non-polluted systems were located in the Sierra Madre Range of the Rocky Mountains, Carbon County, southeast Wyoming, U.S. (Figure 1). The mine-impacted system was in the lower reaches of Haggarty Creek (http://www.wcei.org/wyoming/), which has been influenced by effluent from the Ferris-Haggarty copper mine. The non-polluted system is located in the upper reaches of Big Sandstone Creek, which is a stream that runs parallel to Haggarty Creek but does not receive effluent from the copper mine (Figure 1). Both Haggarty and Big Sandstone Creeks are similar in physical and landscape characteristics, so they are classified as being in the same ecoregion, Southern Rockies (Omernik 1987, 1995). Both streams are within a montane biogeographic zone, characterized by mixed conifer/aspen forest, and are bordered by riparian and wetland vegetation. Such streams are rather narrow and shallow, have relatively cool temperatures, and low discharge rates. Substrates for both streams were mostly gravel but there were some sands and a few cobbles.

Four sampling sites were established at riffles on Haggarty Creek (HC-1, HC-2, HC-3, and HC-4), with one (HC-5) being located at riffles on Battle Creek West Fork just after the confluence of Haggarty Creek and Lost Creek (Figure 1). The first site on Haggarty Creek, HC-1, was situated just upstream from where mine effluent flows into the stream while the others (HC-2, HC-3, HC-4, and HC-5) were located at various points down-

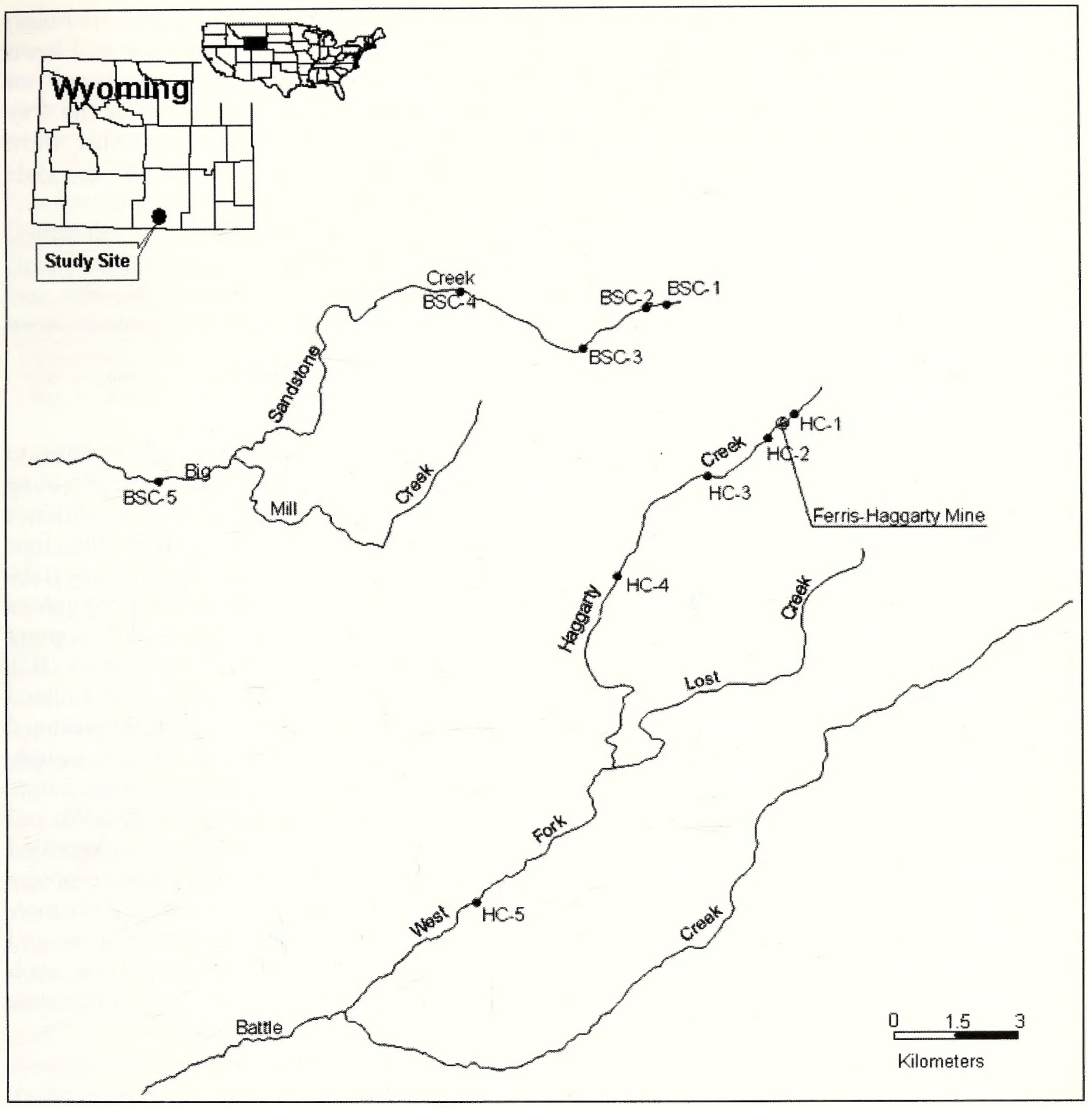

Figure 1. Map of the sampling sites that were located on Haggarty Creek (polluted stream), Big Sandstone Creek, and Battle Creek West Fork (non-polluted stream); Sierra Madre Range of the Rocky Mountains, southeast Wyoming, U.S.

stream. We note here that the site HC-2 was the nearest site downstream from where effluent flows into Haggarty creek, and site HC-5 was the farthest. Haggarty Creek waters are impacted by effluents at all sites except HC-1. Five additional sites were established at riffles on the non-polluted stream, Big Sandstone Creek (BSC-1, BSC-2, BSC-3, BSC-4, and BSC-5) (Figure 1).

Sample Collection

At each site, 8 transects were visually laid out, with 4 being randomly chosen for Surber sampling and the remainder being assigned for D-net sampling. Samples were collected during the summer (from 27 June to 23 July) and autumn (from 22 August to 20 September) in 2001 and 2002.

All Surber and D-net samples were consistently collected in specific ways by one operator. The Surber sampler (area 0.092 m^2, mesh aperture 0.28 mm) was used as described by Surber (1937). Briefly, this entailed placing the Surber frame on a randomly selected location of the stream substrate. Large stones were picked out and scrubbed

into the collecting net to remove pupae and other attached macroinvertebrates. Then the substratum was vigorously disturbed so the current would carry sediment, organic matter, and macroinvertebrates into the net. Collecting was stopped when we reached a substratum depth of 10 cm or encountered solid rock, below which no benthic macroinvertebrates would live.

The D-net (30.5 cm by 16.5 cm) was fitted with a net that had a mesh aperture of 0.9 mm; this aperture size was chosen to prevent macroinvertebrates and other material from backwashing out of the net. We used the D-net by placing it on the streambed and disturbing the substrate immediately upstream by vigorous kicking. The current then carried sediment, organic matter, and dislodged macroinvertebrates into the net. Once a location had been sampled, the net was moved sideways to the adjacent location and that substrate was disturbed by vigorous kicking. One D-net sample was collected by continuously kicking the substrate and moving the net sideways for 5 min; a 5 min sampling time was chosen based on results from a preliminary sampling effort and from suggestions found in the literature (e.g., http://www.neihp.org/documents/mill/mill_eco_health_report.pdf, and http://www.ongov.net/WEP/wepdf/we152002TribMacroinvMonitor.pdf).

At streamside, all collected material was washed from the nets, with water, into a large, deep tray. After washing, the sampling devices were carefully examined and organisms found clinging to the nets were removed and placed into the tray. Material in the tray was then washed through a 250-μm sieve; this sieve retained all organisms captured in the nets of both sampling devices. Material retained in the sieve was placed into plastic bottles containing 95% ethanol; we made sure that all material found in the sieve was transferred to the bottles. Plastic bottles were transported to the laboratory for sorting.

In the laboratory, each sample was sorted by evenly placing all collected material in a shallow white tray, taking care to transfer all material from the bottle to the tray. Organisms were then removed from the tray, beginning at one side and working systematically towards the opposite. After organisms had been removed from the tray, we reexamined the remaining material (sand, pebbles, sticks, etc.) to make sure that none had been missed. Occasionally, when we found an overlooked organism, all material in the tray was reexamined. When all organisms were extracted, they were transferred to vials containing 70% ethanol.

All collected organisms were identified either to family (some) or genus (most) (*taxa*) using the taxonomic keys of Merritt and Cummins (1996). Identified organisms were counted and recorded.

Variables Measured

Metrics used in this study are sensitive to effluent from the Ferris-Haggarty copper mine (Wu 2006). They included the Shannon-Weiner index and the random runs value plus four measures of richness; Ephemeroptera taxa richness, Ephemeroptera/Plecoptera/Trichoptera taxa richness, total taxa richness, and Plecoptera taxa richness. The Shannon-Weiner Index (H'), regarded as a measure of diversity, was calculated as $H' = -\Sigma P_i \ln (P_i)$, where P_i = the proportional abundance of the ith taxon in the sample (Magurran 2004). The random runs value, regarded as another measure of diversity (Wu and Legg unpublished), was calculated as described by Wu and Legg (2007). Finally, Ephemeroptera taxa richness, Plecoptera taxa richness, Ephemeroptera/Plecoptera/Trichoptera taxa richness, and total taxa richness were calculated for each sample by summing the number of taxonomic groups that belong to each metric.

Average metric values from the D-net were computed for each site, each time it was visited. Likewise, average metric values from the Surber sampler were computed for each site, each time it was visited. In all, we computed 39 averages for each metric using each sampling device (i.e., 39 data points) except for the random runs value, for which there were 38 data points.

Statistical Analyses

Simple linear regressions were attempted to determine if average metric values calculated from D-net samples (y) were significantly ($\alpha = 0.05$) and positively related to average metric values calculated from Surber samples (x). One requirement for estimating regression coefficients (y-intercept and slopes) via ordinary least squares is that values for the x

Table 1. Simple linear regression models and effective ranges for relating average D-net values to average Surber sampler values, using six selected metrics, based on samples taken from Haggarty Creek and Big Sandstone Creek in the summer and autumn of 2001 and 2002 (n = 39 except for random runs value, for which n = 38, 4 samples per average); southeast Wyoming, US.

Metric	Simple linear regression models	r^2	Predictive range for Surber sampler	Predictive range for D-net
Ephemeroptera taxa richness	$\hat{y} = 0.208 + 1.050^{a} \times$ Surber	0.96	0.0–7.5	0.0–8.5
Plecoptera taxa richness	$\hat{y} = 0.065 + 0.974^{a} \times$ Surber	0.91	0.0–8.0	0.0–7.8
EPT† taxa richness	$\hat{y} = 0.613 + 1.060^{a} \times$ Surber	0.97	0.0–21.8	0.0–22.8
Total taxa richness	$\hat{y} = -0.081 + 1.099^{a,b} \times$ Surber	0.97	0.3–31.0	0.3–31.8
Shannon-Weiner Index	$\hat{y} = 0.157 + 0.946^{a} \times$ Surber	0.89	0.0–2.7	0.0–2.7
Random runs value	$\hat{y} = 0.203 + 0.761^{a,b} \times$ Surber	0.61	0.1–0.9	0.1–0.9

† Ephemeroptera/Plecoptera/Trichoptera

[a] Slope is significantly (α = 0.05) different from 0.0 ($P < 0.0001$; t-test).

[b] Slope is significantly different from 1.0 (total taxa richness: $P = 0.0056$; random runs value: $P = 0.0205$; t-test).

variable(s) are known exactly or with much less error (i.e., variation) than values for the y-variable. As both x and y in this study were estimates with equal variation, we computed the regression coefficients via Bartlett's (1949) method, which calculates the linear statistical function (i.e., the y-intercept and slope) when there is variation in both x and y. Using this method, we wished to test the null hypothesis that the slope was equal to 0.0 versus the alternate that it was not. If we rejected the null and accepted the alternate, we then tested another null hypothesis that the slope was equal to 1.0 versus the alternate that it was not. Hypotheses were assessed using t-tests. Computations for Bartlett's method (and t-tests) were facilitated through use of a computer program (Legg 1986, http://w3.uwyo.edu/~dlegg/ncci.html). P values for t-tests were calculated using the PROBT function of the Statistical Analysis System (SAS Institute., Cary North Carolina, ver. 9.0).

RESULTS

Results from the regressions indicated that average values for all metrics, when using the D-net, were linearly related to average values derived from using the Surber sampler (Table 1). Overall, regressions for the four richness metrics plus the Shannon-Weiner Index (Figures 2–6) were strong, having r^2 values that were greater than 0.8 (Table 1). The regression for random runs value was moderately strong, having an r^2 of 0.61 (Figure 7).

Slopes for all regressions were positive and significantly different from 0.0. In addition, slopes of four regressions were not different from 1.0 (Table 1). For those slopes that were different from 1.0, that for total taxa richness was greater than 1.0 while that for random runs value was less than 1.0 (Table 1).

DISCUSSION

Relating measures derived from one sampling method to measures derived from another is done for several reasons. In integrated pest management, practitioners wish to determine if population estimates derived from using a simpler and quicker sampling method can be used in lieu of those derived from using a standard but more cumbersome sampling method (Gerrard and Chiang 1970; Wilson and Room 1983; Schaalje and Butts 1992). Also in integrated pest management, relating measures derived from one sampling method to measures derived from another can be done when the results from predictive population models are in scales that cannot be used in pest management; however, application of a simple function may convert those values to scales that can be used in pest management (Legg and Brewer 1995). In climate analysis, relating temperatures that are taken in one location to temperatures that are taken at nearby 'highly correlated' locations is done to adjust for non-climate related factors such as relocating a reporting station, changing instrumentation, and urbanization (Peterson et al. 1998; Allen and DeGaetano 2000).

Given the results of this study, it may be possible to relate some metric values derived from using one sampling method to metric values derived from using another when both are operated at the same sites and times.

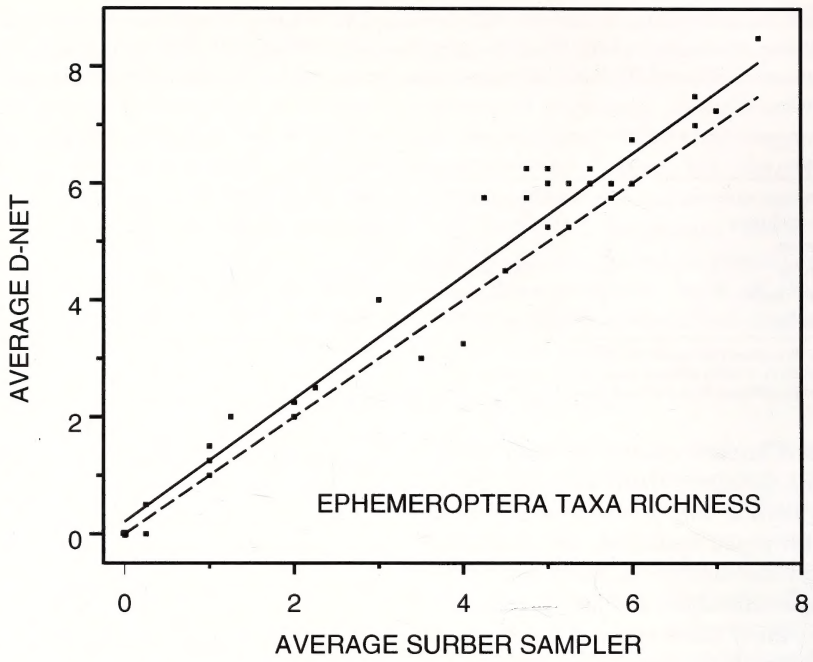

Figure 2. Simple linear regression (solid line) of average Ephemeroptera taxa richness estimated from D-net captures regressed on average Ephemeroptera taxa richness estimated from the Surber sampler; samples were taken from both polluted and non-polluted streams, southeast Wyoming. Dotted line represents the line of unity (i.e., y-intercept = 0 and slope = 1.0).

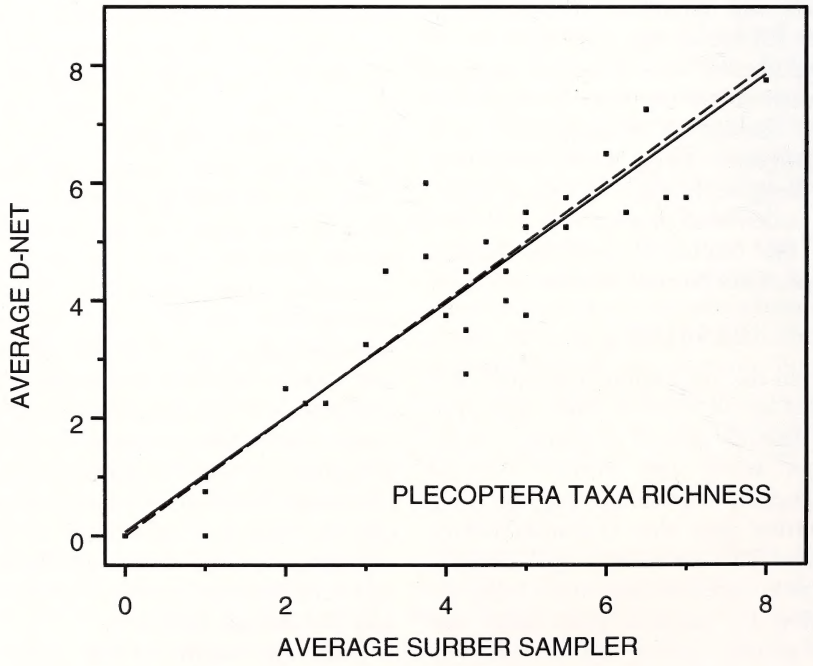

Figure 3. Simple linear regression (solid line) of average Plecoptera taxa richness estimated from D-net captures regressed on average Plecoptera taxa richness estimated from the Surber sampler; samples were taken from both polluted and non-polluted streams, southeast Wyoming. Dotted line represents the line of unity (i.e., y-intercept = 0 and slope = 1.0).

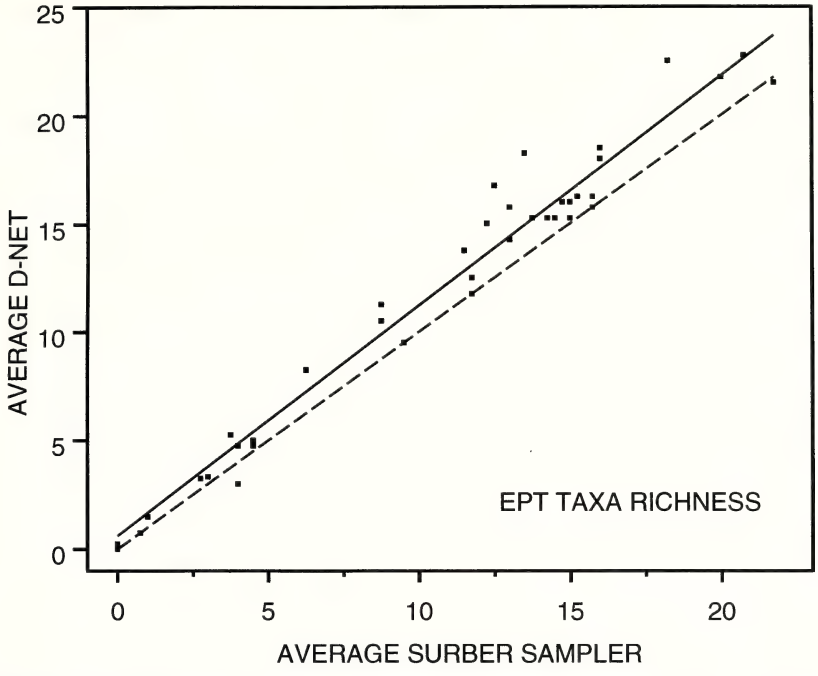

Figure 4. Simple linear regression (solid line) of average Ephemeroptera/Plecoptera/Trichoptera (EPT) taxa richness estimated from D-net captures regressed on average EPT taxa richness estimated from the Surber sampler; samples were taken from both polluted and non-polluted streams, southeast Wyoming. Dotted line represents the line of unity (i.e., y-intercept = 0 and slope = 1.0).

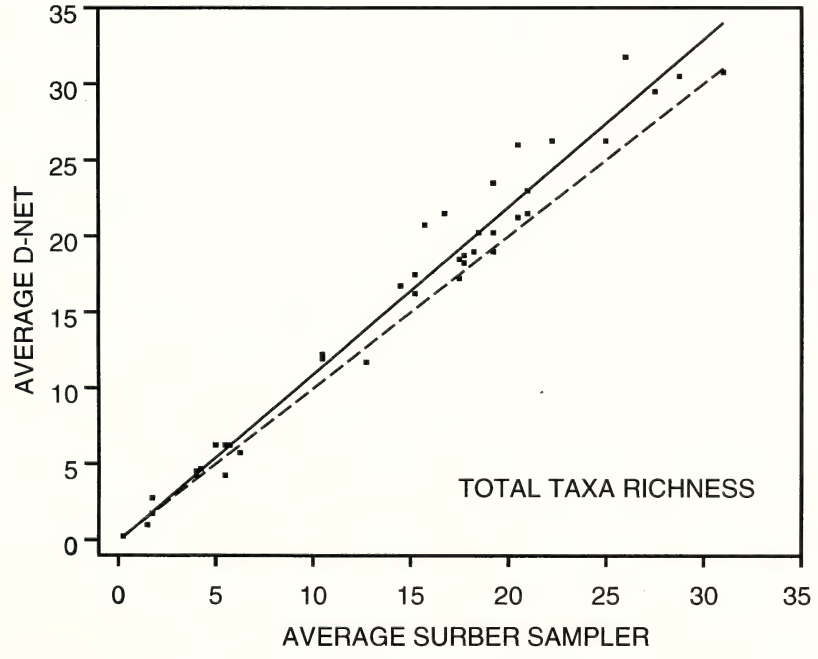

Figure 5. Simple linear regression (solid line) of average total taxa richness estimated from D-net captures regressed on average total taxa richness estimated from the Surber sampler; samples were taken from both polluted and non-polluted streams, southeast Wyoming. Dotted line represents the line of unity (i.e., y-intercept = 0 and slope = 1.0).

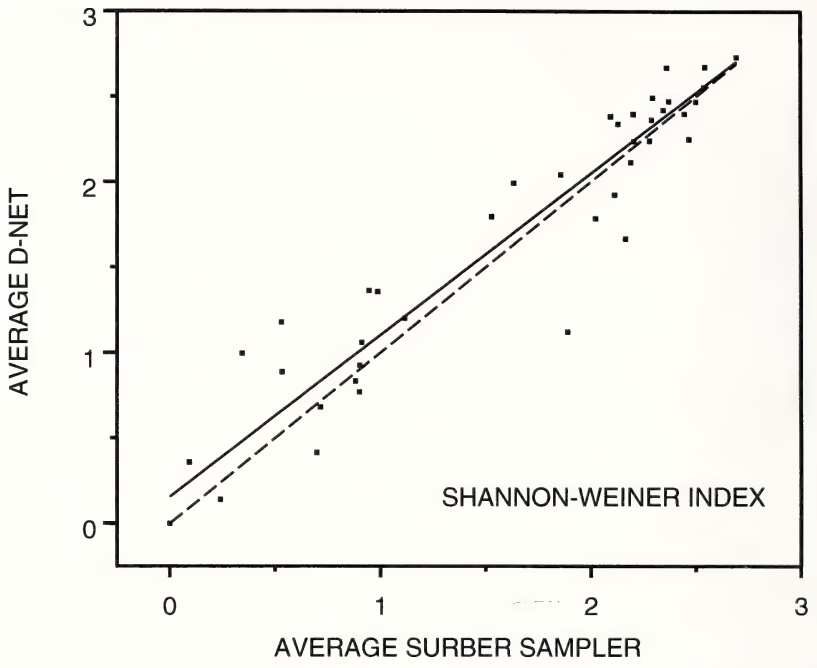

Figure 6. Simple linear regression (solid line) of average Shannon-Weiner Index estimated from D-net captures regressed on average Shannon-Weiner Index estimated from the Surber sampler; samples were taken from both polluted and non-polluted streams, southeast Wyoming. Dotted line represents the line of unity (i.e., y-intercept = 0 and slope = 1.0).

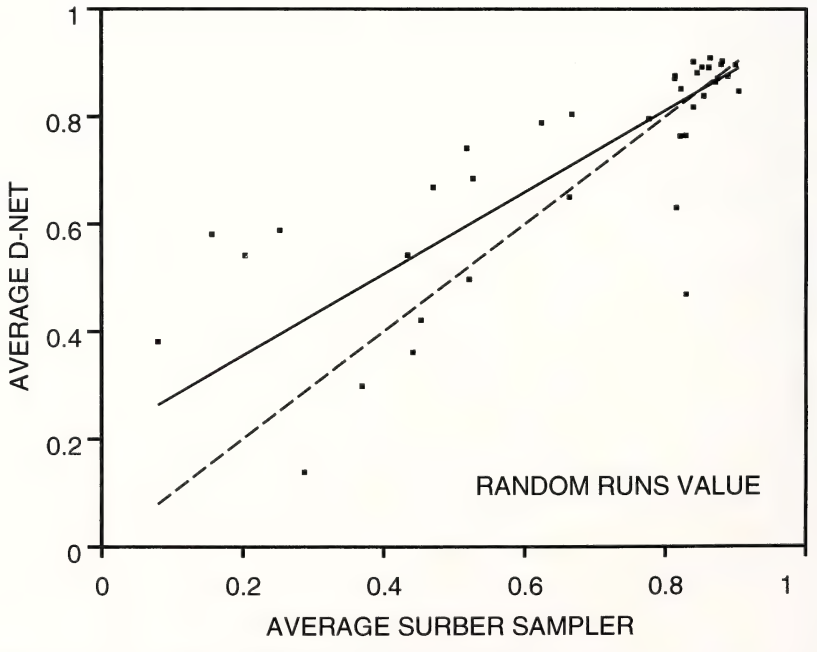

Figure 7. Simple linear regression (solid line) of average Random Runs Value estimated from D-net captures regressed on average Random Runs Value estimated from the Surber sampler; samples were taken from both polluted and non-polluted streams, southeast Wyoming. Dotted line represents the line of unity (i.e., y-intercept = 0 and slope = 1.0).

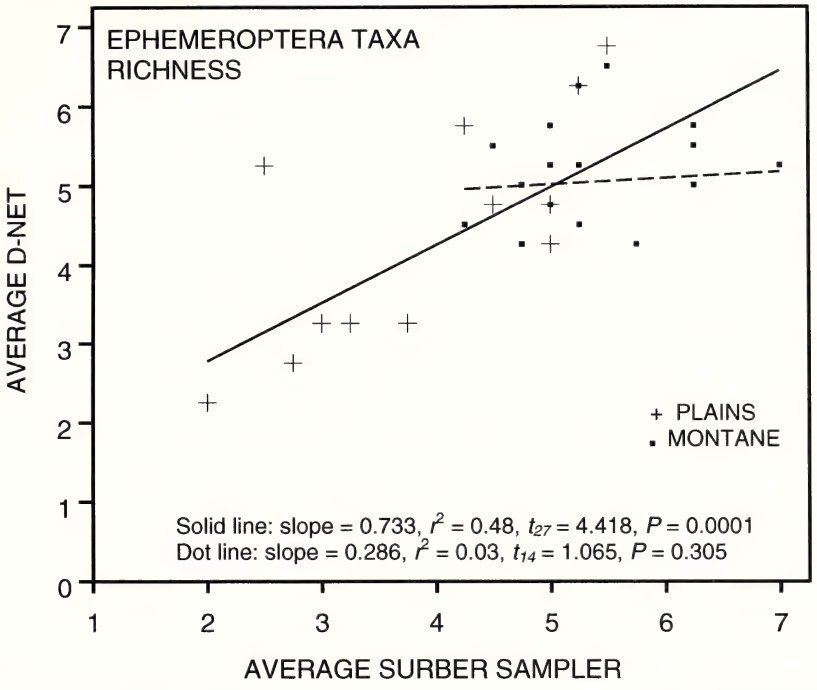

Figure 8. Simple linear regression of average Ephemeroptera taxa richness estimated from D-net captures regressed on average Ephemeroptera taxa richness estimated from the Surber sampler; samples were taken from both Medicine Bow River and Rock Creek, southeast Wyoming. Solid line represents the significant, positive relation for data that were collected over two biogeographical zones (montane and plains); Dotted line represents the insignificant relation for data collected just from the montane biogeographical zone.

When testing to determine if such relations exist, we suggest some considerations. First, it is important to follow good quality assurance/quality control (QA/QC) practices; these ensure high quality of data, which leads to confidence in conclusions drawn from quantitative studies. In this study, we made sure that QA/QC was addressed *prior* to sample collection by developing the sampling protocols and then practicing those protocols until they could be performed consistently and correctly. Then, both *during* and *after* sample collection, we meticulously double-checked the sampling devices, sieves, collecting bottles, and sorting trays for overlooked material. Second, a sufficient range in metric values must be realized to parameterize linear statistical functions between any two sampling methods. Figure 8, which contains two regression 'lines' relating average Ephemeroptera taxa richness values for D-net to average Ephemeroptera taxa richness values for Surber sampler, shows why. Each regression was performed on data (unpublished) that were collected from two non-polluted streams (Medicine Bow River and Rock Creek) that occur in an adjacent mountain range (Snowy Range, Albany County). One regression was performed on data collected from portions of those streams that traversed the montane biogeographical zone; this regression had a slope (0.286), which was not different from zero (t_{14} = 1.065, P = 0.305, r^2 = 0.03). This happened because the range in Ephemeroptera taxa richness values (4.25–7.0) was too narrow to estimate the slope. However, when the regression was performed on data collected over two biogeographical zones (montane and plains), the range in Ephemeroptera taxa richness values doubled (1.5–7.0) and a significant, positive slope was revealed (slope = 0.733, t_{27} = 4.418, P = 0.0001, r^2 = 0.48, Figure 8). Third, linear statistical functions that express values of a metric, derived from using one sampling method, as values derived from using another, should not be applied to values that lie outside the range of values represented in the original data set (predictive

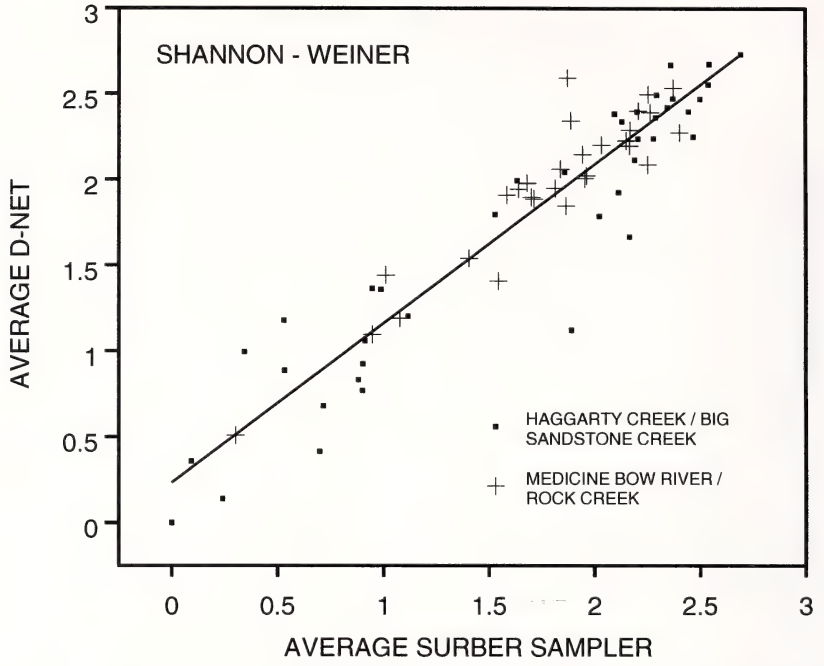

Figure 9. Simple linear regression ($\hat{y} = 0.234 + 0.927 \times$ Surber, $r^2 = 0.88$) between average Shannon-Weiner values estimated from D-net captures and average Shannon-Weiner values estimated from Surber sampler; samples were pooled over two locations (Haggarty Creek/Big Sandstone Creek and Medicine Bow River/Rock Creek), representing two mountain ranges located in southeast Wyoming.

range). Doing so invites uncertainty when interpreting the results (extrapolation). Reporting the predictive range of values in the original data set (Table 1) provides what is needed to avoid extrapolation. Finally, linear statistical functions that convert values of a metric, derived from using one sampling method, into values derived from using another can be tested for transportability among locations and regions. This can be done in a number of ways, two of which we discuss here.

The first is when there are two data sets representing two locations or regions. Each data set contains the results from using two sampling methods. A linear statistical function can be developed for each metric in each data set. The F test of Weisberg (1980) can then be used to test the null hypothesis that these two functions have the same y-intercept and slope versus the alternate that they do not. If the intercepts and slopes are the same, one function can be calculated and used for both locations/regions. Use of the F test with Bartlett's method is described by Lockwood et al. (1990).

For example, regressions of average Shannon-Weiner values derived from using D-net to average Shannon-Weiner values derived from using Surber sampler were calculated for the Haggarty Creek-Big Sandstone Creek location (Table 1) and Medicine Bow River-Rock Creek location (unpublished) ($\hat{y} = 0.394 + 0.873 \times$ Surber, where $\hat{y}$ is the predicted average Shannon-Weiner value for D-net; $n = 29$ data points). The F test for identical intercepts and slopes indicated that intercepts and slopes were the same ($F_{2,64} = 6.46$, $P = 0.2353$), so a combined function was calculated for the two locations ($\hat{y} = 0.234 + 0.927 \times$ Surber, $r^2 = 0.88$, $t_{66} = 19.9$, $P < 0.0001$, Figure 9).

The second is a model validation technique that can be used when a data set exists from just one location (or region); data from the second location are not available. Instead, a linear statistical function exists for that second location. In this case, the linear statistical function of the second location can be used to predict values for the y variable (predicted y values) of the first by using values for the x variable, also from the first location. Observed y values can then be regressed on predicted y values using ordinary least squares to check

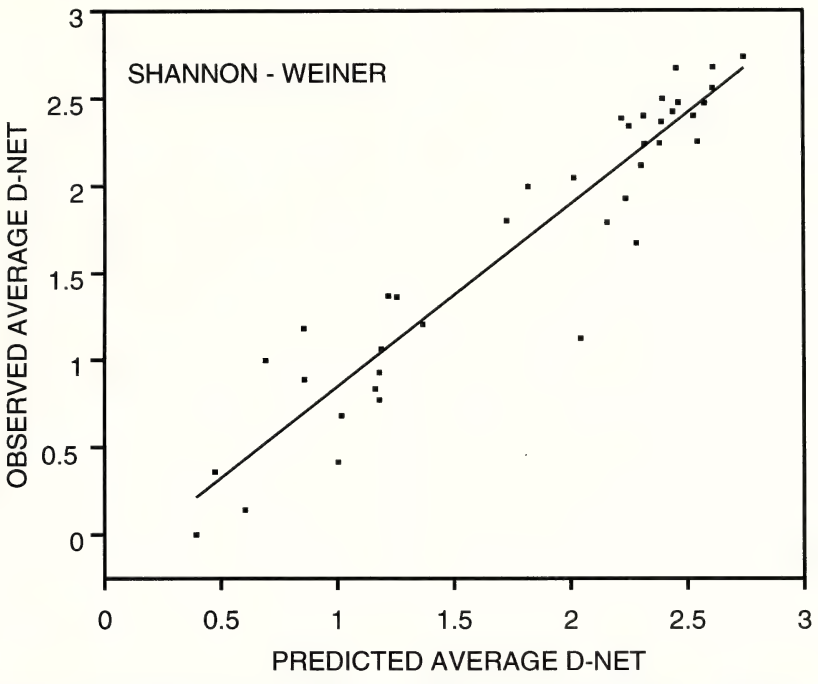

Figure 10. For D-net sampling technique, a simple linear relation ($\hat{y} = -0.199 + 1.044 \times$ predicted D-net; $r^2 = 0.89$) exists between observed average Shannon-Weiner values obtained from sampling the Haggarty Creek-Big Sandstone Creek location, Sierra Madre Range, southeast Wyoming, and predicted average Shannon-Weiner values, which were predicted by using a linear statistical function ($\hat{y} = 0.394 + 0.873 \times$ Surber) created to express the relation between average Shannon-Weiner values from using D-net and average Shannon-Weiner values from using Surber sampler in an entirely different location (Medicine Bow River-Rock Creek) in an adjacent mountain range (Snowy Range, southeast Wyoming). Statistical tests indicated that the y-intercept was equal to zero ($t_{37} = -1.7$, $P = 0.097$) and the slope was equal to 1.0 ($t_{37} = 0.74$, $P = 0.464$). Predicting average D-net values for one location by using a linear statistical function from a second can be done to see if the function from that second location is essentially the same as the function from the first, but there is no data available from the second location; just the function is available.

for: 1) a linear response, 2) a y-intercept of 0.0, and 3) a slope of 1.0.

For example, using the Shannon-Weiner linear statistical function from the Medicine Bow River-Rock Creek location ($\hat{y} = 0.394 + 0.873 \times$ Surber), along with the Shannon-Weiner data from the Haggarty Creek-Big Sandstone Creek location, we first predicted average D-net values for the Haggarty Creek-Big Sandstone Creek location (predicted y). Next, observed average D-net values (observed y) from the Haggarty Creek-Big Sandstone Creek location were regressed on corresponding predicted y values to yield the following linear statistical function: observed $y = -0.199 + 1.044 \times$ predicted y ($r^2 = 0.89$). This relation is linear with the y-intercept being equal to 0.0 ($t_{37} = -1.7$, $P = 0.0968$), and the slope being equal to 1.0 ($t_{37} = 0.74$, $P = 0.464$) (Figure 10). The linear statistical function from the Medicine Bow River-Rock Creek location may be used to predict average Surber sampler values (predicted x) for Haggarty Creek-Big Sandstone Creek. Then the observed average Surber sampler values (observed x) could be regressed on the predicted values to see if there is a linear relation, a y-intercept of 0.0, and a slope of 1.0.

Use of Bartlett's or some other method (e.g., Fuller 1987) to estimate the linear statistical function between metric values derived from two sampling methods should be considered, as ordinary least squares tends to underestimate the value of the slope when there is error in both x and y (Lockwood et al. 1990; Schaalje and Butts 1992). However, Bartlett's method does have its limitations. For example, there is no statistical test for the y-intercept. Plus the F test for identical y-intercepts and slopes is not exact.

In summary, it appears that linear statistical functions can be developed to express average metric values, derived from using the D-net, as average metric values derived from using the Surber Sampler (and vice versa), when the D-net and Surber sampler are used in the manner described, for selected metrics, in two southeast Wyoming streams. Transportability of those functions can and should be tested for each metric for potential use in other locations and/or regions. We have presented two ways to test for transportability. One made use of the F test for identical y-intercepts and slopes when there are data from each location. The other made use of a model validation technique when there are data from one location and a linear statistical function from another. Either can be used to test for transportability, but the model validation technique is more versatile as it can be used as a correction factor when linear statistical functions describing the relation between two sampling methods are not the same between locations.

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The Dragonflies and Damselflies (Insecta: Odonata) of the Upper Rockcastle River System, Kentucky, U.S.A.

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ABSTRACT

A survey of the adult dragonfly and damselfly (Odonata) fauna of streams in the upper Rockcastle River system, Kentucky, was conducted during 2002–2003. Twenty-seven species were collected, resulting in 31 new county records for Jackson, Laurel, and Rockcastle counties and the extension of the Kentucky flight season for six species. The 27 species collected during this study represent 18 percent of the odonate species currently known from Kentucky.

KEY WORDS: Odonata, dragonflies, damselflies, Kentucky, Rockcastle River, Horse Lick Creek

INTRODUCTION

The Commonwealth of Kentucky contains 152 species of dragonflies and damselflies, nearly one-third of the Odonata species known from the United States (Abbot 2007). Resener (1970), in the last published annotated list of Kentucky's Odonata, showed that the eastern and western counties of Kentucky have been less intensely studied than those in the central portion of the state. Due to the lack of published records from southeastern Kentucky, it was decided that a survey would be conducted of the Odonata of streams in the upper Rockcastle River system, a sixth-order tributary of the Cumberland River (Figure 1).

Previous studies have shown a large diversity of aquatic organisms in the upper Rockcastle River system including 54 mayfly species (Randolph and McCafferty 1998), 37 stonefly species (Tarter et al. 2006), 17 crayfish species (Taylor and Schuster 2004), 39 freshwater mussel species (Cicerello 1992, 1993, 1994), 64 native fish species (Burr and Warren 1986), and 121 species of aquatic and semi-aquatic plants (Beal and Thieret 1987). Williamson (1905) provided the first published records of Odonata from this area when he collected nine species from the Rockcastle River below Livingston, Kentucky (Site 1 in Table 1). Resener (1970) recorded 21 species from Jackson, Laurel and Rockcastle counties and 50 species are currently known from this area (Abbot 2007). Twenty of the odonate species now known from the upper Rockcastle River system were first collected by the Kentucky State Nature Preserves Commission (KSNPC) (E. Laudermilk, KSNPC, pers. comm., 3 Mar 2004) which currently monitor five odonate species (*Ophiogomphus howei* Bromley, *O. mainensis* Packard in Walsh, *Stylurus notatus* (Rambur), *S. scudderi* (Selys), *Nannothemis bella* (Uhler)) in this area (KSNPC 2007).

MATERIALS AND METHODS

Study Area

The Rockcastle River system drains 1976 km^2 in Pulaski, Rockcastle, Jackson, Laurel and Clay counties in southeastern Kentucky. The mainstem of the Rockcastle River flows southward for 86.9 km before entering the Cumberland River 14.8 km downstream of Cumberland Falls (Harker et al. 1980). This system is located entirely within the northernmost area of the Southwestern Appalachian ecoregion (Woods et al. 2002) with surface geology composed of Pennsylvanian age shale and siltstone and Mississippian age limestone (Harker et al. 1980). Nearly

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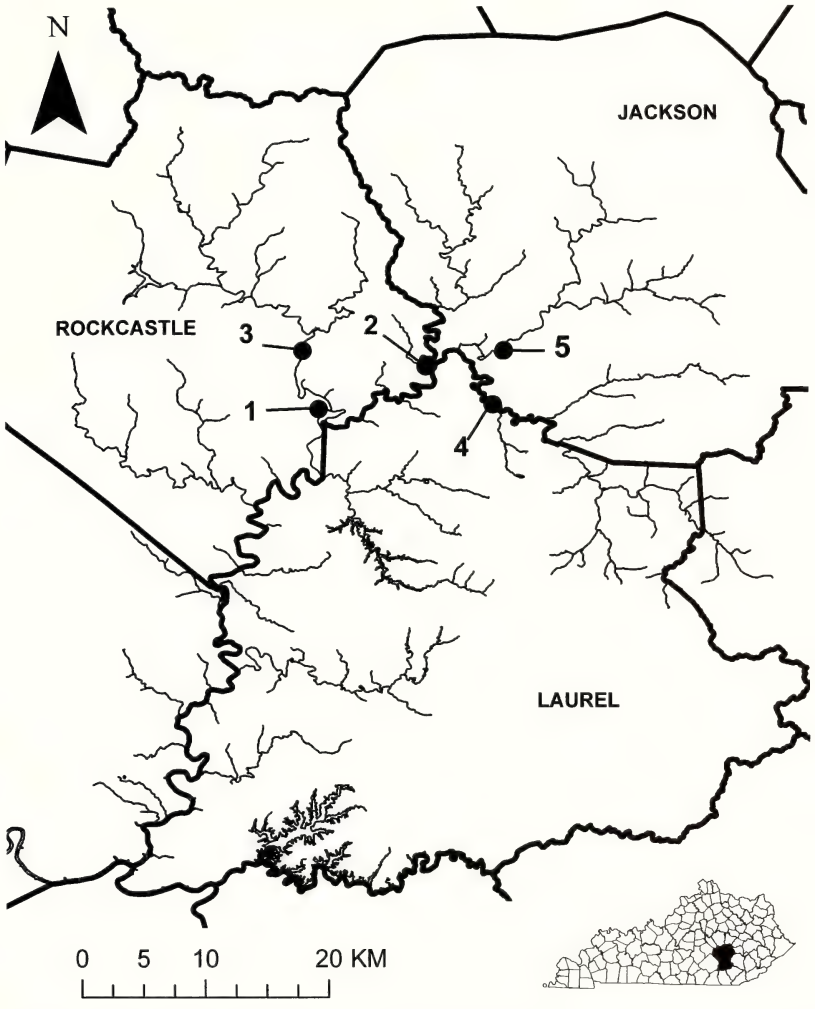

Figure 1. Location of sites sampled in 2002–03 in the upper Rockcastle River drainage. Site numbers correspond to those listed in Table 1.

25.5 km of the Rockcastle River have been designated as a Kentucky Wild River due to its high water quality and pristine setting (Kentucky Department of Natural Resources and Environmental Protection 1980). However, 10 stream segments in this system (totaling 59 stream km) are listed on Kentucky's 303(d) list due to impacts from poor agricultural and silvicultural practices, livestock grazing, sewage and septic system discharges, loss of riparian vegetation, and surface mining (Kentucky Division of Water 2007).

Table 1. Location of collecting sites in the upper Rockcastle River system, Kentucky.

Site	Location
1	Rockcastle River, off of US 25S at ford immediately south of Livingston (N 37° 17.58′, W 84° 13.23′)
2	Horse Lick Creek, off of FR 465, 0.5 km NW of the junction of FR 489 and SR 89 (N 37° 19.45′, W 84° 08.54′)
3	Roundstone Creek, off of FR 465 at Sinks, 0.5 km SSE of Mullins in Rockcastle County (N 37° 20.14′, W 84° 13.93′)
4	South Fork of Rockcastle River at Jervis Rd. crossing, approximately 2.77 km N of Cornette (N 37° 17.77′, W 84° 05.60′)
5	Middle Fork of Rockcastle River, off of SR 89, 1.3 km SSW of the junction of SR 89 and SR 2002 (N 37° 20.14′, W 84° 05.11′)

Table 2. Odonata species collected in the upper Rockcastle River drainage in 2002–03. "*" in the "Species" column indicates those species also collected by Payne (1993, 1995). "(+N)" in the "Flight Season" column indicate extensions, in days, to Kentucky's flight season for that species. Numbers in the "Sites" column correspond to the site numbers listed in Table 1. "X" represents a new county record; "x" indicates a previous county record.

Species	Flight Season	Sites	Laurel	Jackson	Rockcastle
CALOPTERYGIDAE					
Calopteryx maculata (Beauvois)*	23 May–24 September	1 2 3 4 5	x	x	x
Hetaerina americana (Fabricius)*	31 July–1 Oct. (+8)	1 4 5	x	X	x
COENAGRIONIDAE					
Argia apicalis (Say)	15 July	1	X		x
Argia fumipennis violacea (Hagen)*	10 June–29 September (+5)	1 2 4	X	X	x
Argia moesta (Hagen)*	10 June–24 September	1 2 3 4 5	x	x	x
Argia tibialis (Rambur)*	10 June–31 July	2 3 4 5	x	x	x
Argia translata Hagen in Selys*	26 June–10 September	1 2 3 4 5	x	X	X
Chromagrion conditum Hagen in Selys	2 June	2	x		X
Enallagma exsulans (Hagen)*	10 June–16 August	1 2 4 5	X	x	x
Ischnura hastata (Say)	17 June	1	X		X
Ischnura posita (Hagen)*	23 May–26 July	3 4 5	X	X	x
AESHNIDAE					
Anax junius (Drury)	22 October	4	X	X	
Basiaeschna janata (Say)*	26 April–23 May	3 5		X	x
Boyeria vinosa (Say)*	26 July–1 October	1 2 3 4 5	x	x	x
CORDULEGASTRIDAE					
Cordulegaster maculata Selys	30 May	5		x	
CORDULIIDAE					
Helocordulia uhleri (Selys)	26 April–30 May	4 5	X	X	
Somatochlora linearis (Hagen)*	13 Sept.	3			X
GOMPHIDAE					
Dromogomphus spinosus Selys*	8 July–17 September	1 2 3 4 5	x	x	x
Gomphus lineatifrons Calvert*	13 May–11 July (+14)	1 5	x	x	x
Gomphus lividus Selys*	14 May–23 May	3 5		X	x
Hagenius brevistylus Selys*	17 June–3 September	1 3 5	x	x	x
Stylogomphus albistylus (Hagen in Selys)*	23 May (+7)–24 June	4 5	X	X	
LIBELLULIDAE					
Erythemis simplicicollis (Say)*	8 July–10 September	3 4 5	X	X	X
Plathemis lydia (Drury)*	10 June–3 July	3 5		X	X
Sympetrum semicinctum (Say)	31 July (+25)	5		X	
MACROMIIDAE					
Didymops transversa (Say)*	26 April–1 May	4 5	X	X	
Macromia alleghaniensis Williamson*	27 August (+8)	4	X	X	

Odonate Collections

Five collecting sites were chosen in the upper Rockcastle River drainage based on their distribution throughout the system, accessibility, and stream order (Figure 1, Table 1). Site one was located on a sixth-order stream, sites two and three were located on fourth-order streams and sites four and five were located on fifth-order streams. Harker et al. (1980) showed that medium order streams such as these have a higher average number of odonate species than lower order streams in the Upper Cumberland River Basin of Kentucky.

Sampling was conducted between June and October 2002 and March and September 2003 with each site visited every two to three weeks. Adult odonates were collected with a large aerial net, placed in glassine envelopes, and immersed in acetone overnight (Needham et al. 2000). Identifications were made with Needham et al. (2000), Westfall and May (1996), and Glotzhober and McShaffrey (2002). Specimens are currently held in the personal collection of the first author.

RESULTS AND DISCUSSION

Twenty-seven species representing two families of Zygoptera (Calopterygidae and Coenagrionidae) and six families of Anisoptera (Aeshnidae, Cordulegastridae, Corduliidae, Gomphidae, Macromiidae and Libellulidae) were collected and identified from the upper Rockcastle River system (Table 2). Diversity

was greatest within the family Coenagrionidae (nine species), which accounted for 33 percent of the total number of species collected. The next most diverse families were Gomphidae (five species, 18.5 percent), Aeshnidae and Libellulidae (three species, 11.1 percent each). The greatest odonate diversity was seen at site five (Middle Fork of the Rockcastle River) which had 20 species. Sites two (Horse Lick Creek) and three (Roundstone Creek) had the fewest with 10 and 11 species, respectively.

A total of 31 new county distribution records were recorded for the upper Rockcastle River system during this study. Fourteen new species were recorded from Jackson County, 11 from Laurel County, and six from Rockcastle County (Table 2). Six odonate species (*Ischnura hastata* (Say), *Somatochlora linearis* (Hagen), *Erythemis simplicicollis* (Say), *Plathemis lydia* (Drury), *Sympetrum semicinctum* (Say), and *Didymops transversa* (Say)) had not previously been collected from any of the counties comprising the upper Rockcastle River system.

The length of the Kentucky flight season for six species was extended by five to 25 days (E. Laudermilk, KSNPC, pers. comm., 3 March 2004). The 27 species collected during this study represent 18 percent of the 152 odonate species currently known from Kentucky (Abbot 2007).

This study represents only the second season-long survey of Odonata to be published in Kentucky. Payne's (1993, 1995) study of Buck Creek, in nearby Pulaski County, recorded 32 odonate species over the course of two collecting seasons. Twenty of the species collected by Payne were also collected during the current study (Table 2).

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Populations of Lady Beetles and Lacewings in Sweet Corn Using 2-Phenylethanol Based Benallure® Beneficial Insect Lures

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ABSTRACT

Sweet corn was grown in 9.3 m^2 replicated plots following National Organic Program Standards. Plots were baited with Benallure® beneficial insect lures containing 2-phenylethanol or were left as non baited controls. Beneficial insects were sampled weekly during silking using 232 cm^2 yellow sticky traps placed at ear height directly in the plot center and 0.9 m from plot center in each cardinal direction. Pink lady beetles [*Coleomegilla maculata* (DeGeer)], multicolored Asian lady beetles [*Harmonia axyridis* (Pallas)], and green lacewings [*Chrysoperla carnea* (Stephens)], were the predatory insects collected on the traps. The pink lady beetle was the most abundant predator caught. Multicolored Asian lady beetles and green lacewings were caught in low numbers during both years of the study. However, multicolored Asian lady beetles were more abundant in Benallure baited plots than non baited plots early during anthesis in 2006. Benallure lures did not attract pink lady beetles or green lacewings during either year of the study.

KEY WORDS: Pink lady beetle, multicolored Asian lady beetle, green lacewing, sweet corn, Benallure®

INTRODUCTION

Important insect pests in sweet corn (*Zea mays* L. *var rugosa*) fields include corn earworm [*Helicoverpa zea* (Boddie)], European corn borer [*Ostrinia nubilalis* (Hübner)], southwestern corn borer [*Diatreae grandiosella* Dyar], and fall armyworm [*Spodoptera frugiperda* (J.E. Smith)]. These and other sweet corn pests typically are controlled using broad spectrum insecticides and genetically engineered crops containing *Bacillus thuringiensis* Berliner crystal proteins that impart resistance to these pests. Concerns, however, regarding negative effects of broad spectrum insecticides and genetically engineered crops on biodiversity and non-target beneficial insect predators and parasitoids have been expressed. Methods of pest management that are economical and minimize the use of synthetic chemical insecticides while leading to high quality produce with minimal insect damage are needed.

The use of predatory insects such as lady beetles (Coleoptera: Coccinellidae) and lacewings (Neuroptera: Chrysopidae) to suppress populations of pest insects has long been accepted and their importance in pest population regulation recognized. The pink lady beetle [*Coleomegilla maculata* (DeGeer)] is an egg and larval predator of several lepidopteran pest species of sweet corn ears and other crops (Obrycki and Kring 1998; Cottrell and Yeargan 1998; Phoofolo et al. 2001). The multicolored Asian lady beetle [*Harmonia axyridis* (Pallas)] is primarily aphidophagous but also feeds on other small soft-bodied insects (Hodek and Honek 1996; Koch 2003). Similarly, the green lacewing, *Chrysoperla carnea* (Stephens), is a predator of many agricultural insect pests (Tauber et al. 2000).

Conservation biological control encompasses techniques used to increase the activity and density of natural enemies already present in a system. Using semiochemicals to attract and retain beneficial insects is a relatively new field of study. Little work has been done in this area (Khan et al. 2008).

Zhu et al. (1999) found pink lady beetles and green lacewings attracted to 2-phenylethanol, an alfalfa and corn plant volatile. A predator lure based on 2-phenylethanol was developed into an attractant (i.e., Benallure®) for these predaceous insects and patented (Baker et al. 2001) for biologically intensive pest suppression (Obrycki et al. 2001); however, the data on effectiveness in field situations and horticultural crops are scarce. In fact, the only reported field experiment concerning Benallure was conducted in alfalfa in Iowa (Zhu et al. 1999; Baker et al. 2001). Thus, the objective of this experiment was to evaluate Benallure as a predaceous insect

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attractant during silking in late planted sweet corn, grown using National Organic Program Standards.

MATERIALS AND METHODS

Prime Plus® sweet corn seed was planted and grown using National Organic Program Standards at the Kentucky State University Agricultural Research Farm in Franklin County, KY. Nature Safe® fertilizer (10-2-8) was spread at a rate of 335 kg N per ha before planting and incorporation. Sweet corn was planted on 6 June 2004 and on 7 July 2006.

Benallure® lures (MSTRS Technologies, Ames, IA) were purchased from Gardens Alive (Lawrenceberg, IN). Lures did not come with directions for use, however, a photograph of the lure hanging on a corn plant at ear height was observed on their web site.

Gardens Alive provided information on their web site stating that each Benallure lure covered 3 m^2 of crop area. Thus, six sweet corn plots, each measuring 3.7 m wide × 2.5 m long, were subdivided from each of two parallel main plots 9.1 m wide × 61 m long. Each main plot was separated by a 10 m buffer zone that had been mowed. Subplots within each plot were separated by 2 m mowed buffers. For each year of the study, the 12 plots were arranged in a randomized complete block design.

Treatments were either Benallure lure present or no Benallure. Five stakes were driven into each of the plots. The locations were plot center and at each cardinal direction (N, S, E, W) 0.9 m from the center stake. Lures were hung near the silks of the corn plant nearest the center of the plot.

Yellow sticky traps (232 cm^2) in area were used to capture and quantify selected beneficial insects in each plot. Sticky traps were stapled to each stake in each plot at ear height as silks began to emerge. The sticky traps were collected and replaced in the sweet corn plots at weekly intervals on 6 and 14 August 2004 and on 5, 14, and 21 September 2006. Traps were wrapped in Saran™ clear plastic wrap, labeled, and returned to the laboratory for insect identification and enumeration.

Traps were analyzed in the laboratory with the help of a dissecting microscope. Lady beetle species were identified using the picture key of Marshall and Cheung (2002). The number of lady beetles per species and green lacewings present on each sticky trap was recorded. Predator populations in control versus Benallure baited plots were analyzed using ANOVA (SAS Institute 2003). Significance was determined at $P \leq 0.10$.

RESULTS AND DISCUSSION

Pink lady beetles, multicolored Asian lady beetles, and green lacewings were the predatory insects collected on the traps (Figures 1–3). Pink lady beetles were the most abundant predator collected in both 2004 and 2006. There were no significant differences in numbers of pink lady beetles caught among plots having Benallure lures present and those that did not during either year (Figure 1a and Figure 2a). Multicolored Asian lady beetles were captured in low numbers in both years. There was a tendency toward higher numbers of multicolored Asian lady beetles in Benallure baited plots on the 6 August 2004 sampling date, but there was no significant difference (Figure 1b). In 2006, significantly more ($P < 0.10$) multicolored Asian lady beetles were trapped in Benallure baited plots than in non baited plots on 5 September (Figure 2b). The trend was opposite where there were significantly more multicolored Asian lady beetles trapped in non Benallure baited plots on 14 September ($P < 0.10$) and 21 September ($P < 0.05$), respectively. It is unknown why this reversal occurred. Green lacewings were not caught in either baited or non baited plots in 2004 or on 14 and 21 September 2006. There was no significant difference in the abundance of green lacewings between Benallure baited and non baited plots in 2006 (Figure 3).

Benallure lures did not attract significantly more pink lady beetles or green lacewings in baited sweet corn plots even though the active ingredient in the lures, 2-phenylethanol, was shown to attract these insects in laboratory tests (Zhu et al. 1999; Baker et al. 2001) and one field test conducted in alfalfa (Zhu et al. 1999; Baker et al. 2001). This could be due to baited and non baited plots being too close to one another and subsequent saturation of the baited and non baited plot areas with 2-phenylethanol. No significant differences between baited and non baited plots may also have been due to the landscape in which the sweet corn was grown being too complex with

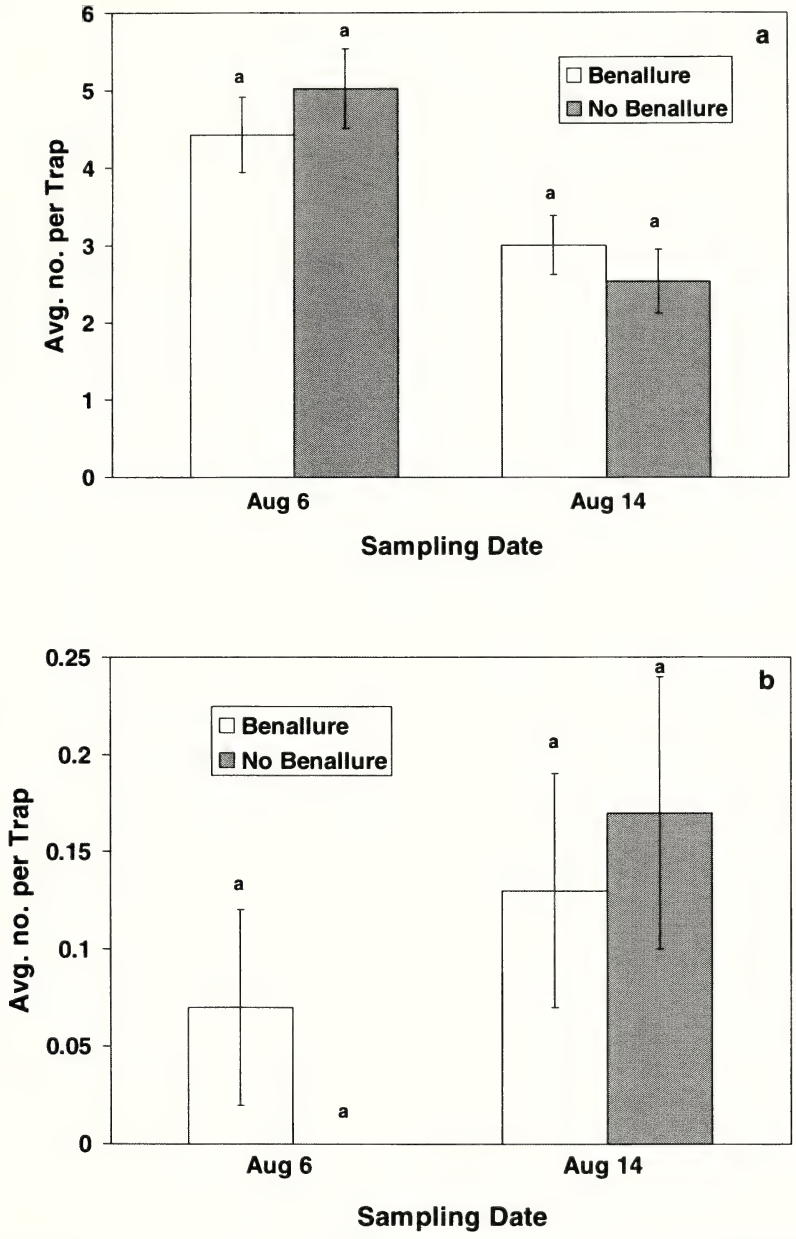

Figure 1. Average number of (a) pink lady beetles and (b) multicolored Asian lady beetles trapped in Benallure baited and non baited sweet corn plots during 2004.

too many olfactory cues present (Lanier 1990). For example, during each year of the study, >75% of all corn rows had pigweed (*Amaranthus* spp.) growing within them. Smooth pigweed (*Amaranthus hybridus*) is neither an alternative food nor an oviposition host of pink lady beetle (Griffin and Yeargan 2002a, b). Its effects on multicolored Asian lady beetle and green lacewing are unknown. Thus, it is unclear what role pigweed and other weeds in the presence of 2-phenylethanol have on the predators. Finally, the rate of emission of the 2-phenylethanol from the dispenser and the duration of its effectiveness may have accounted for no differences between the plots. As temperature increases, the rate of release of volatiles increases rapidly, and thus the release rate is too rapid

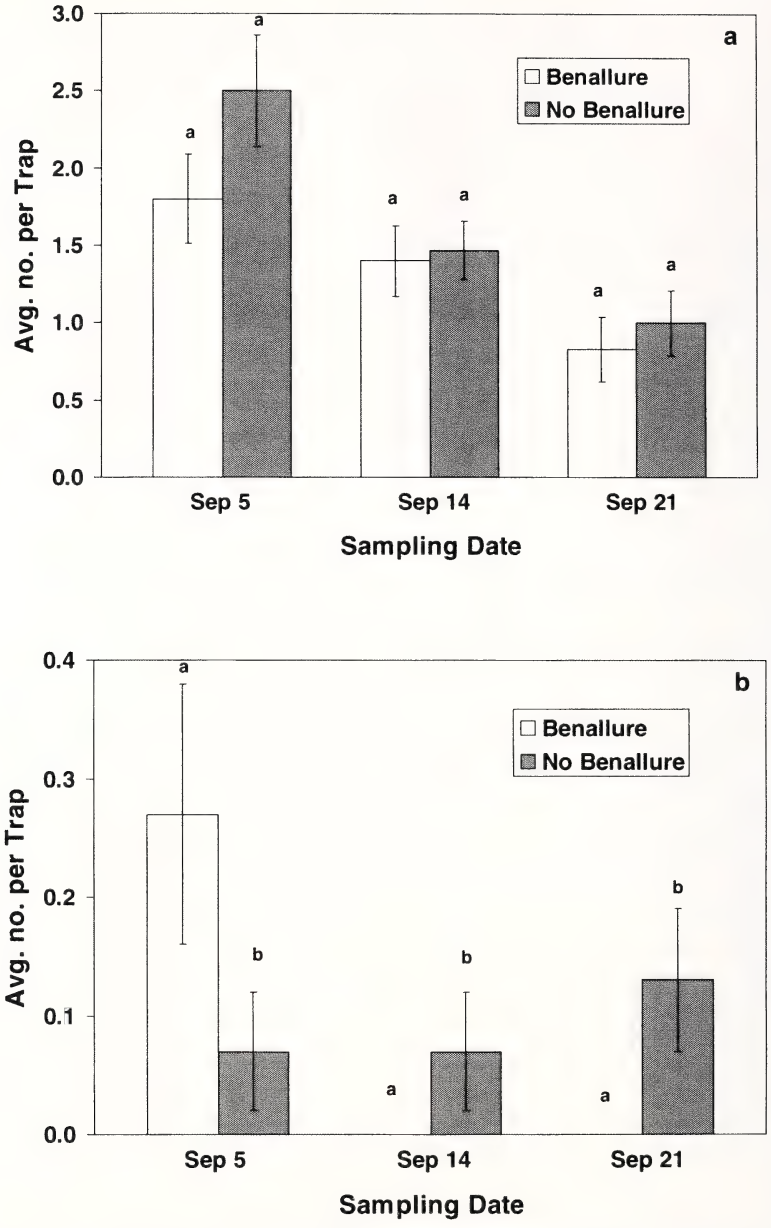

Figure 2. Average number of (a) pink lady beetles and (b) multicolored Asian lady beetles trapped in Benallure baited and non baited sweet corn plots during 2006.

(Leonhardt et al. 1990). These lures may be particularly susceptible to this phenomenon because they were deployed during hot periods in August and September without any covering shielding them from the sun.

Based on the results of these experiments, it was concluded that using Benallure lures in the manner with which they were deployed did not significantly increase the numbers of beneficial lady beetles or lacewings during silking in late planted sweet corn. However, it is believed that more field studies concerning these and other beneficial insect lures should be conducted to determine if they could be employed using different deployment strategies to increase and retain beneficial insect

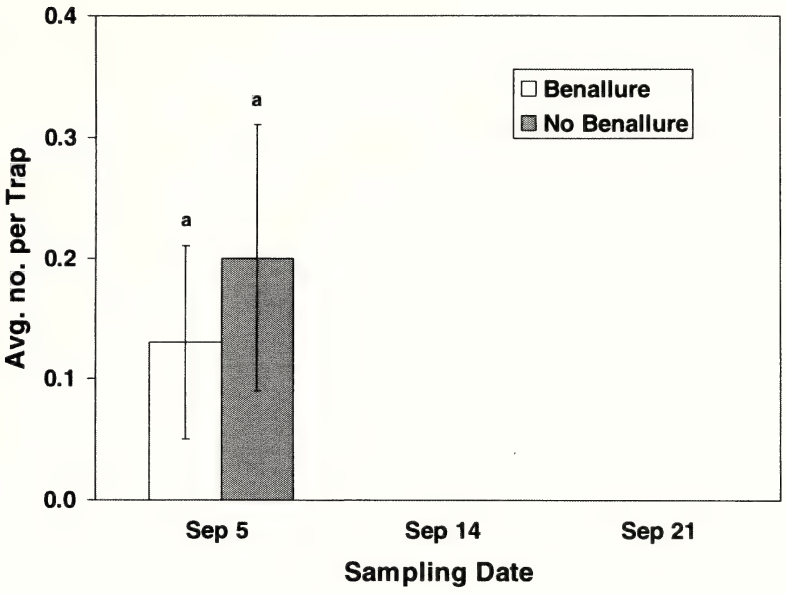

Figure 3. Average number of green lacewings trapped in Benallure baited and non baited sweet corn plots during 2006.

populations in sweet corn and other vegetable crop systems for conservation biological control.

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Consumer Perceptions of Kentucky-Grown Chicken Products in Farmers' Markets

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ABSTRACT

Data were collected via a survey of patrons of farmers' markets in Kentucky to determine consumer perceptions of chicken products. A conjoint experiment was included in the survey that elicited respondent preferences for different chicken product attributes. Results showed that a majority of consumers were willing to buy chickens in farmers' markets, with strong preference towards chicken breast quarters and whole-dressed birds. Certified organic chickens were more popular among Caucasian consumers. Conjoint analysis results indicated that product price and form were more important to consumers than the product's origin or whether or not the chickens were organic.

INTRODUCTION

Kentucky's agricultural landscape consists of many small-scale farmers seeking to be profitable using low-investment enterprises. Small-scale broiler production is one such enterprise where chickens are raised on pastures in bottomless cages so that they can access vegetation, seeds, insects, etc. in addition to prepared feed. This is unlike intensive commercial broiler production where the animals are grown in climate-controlled enclosed buildings without access to a more natural environment (Cunningham 2008). It is obvious that small-scale pastured broiler farming has higher production costs per chicken than large scale intensive farms where cost efficiency is a primary management goal. Cunningham (2009) reported that intensive broiler operations received, on average, $0.40 per bird from integrated chicken processors while Dasgupta and Skelton (2007) used Kentucky pastured poultry data to project a breakeven production cost of $4.67 per bird. Higher costs make much of Kentucky's pastured broilers a higher-priced product, leading to marketing concerns among farmers. Concerns usually result in producers seeking niche markets where they could receive a greater share of retail prices.

Farmers' markets were designed to offer consumers with locally-grown food products. In many cases these outlets have evolved to become sources of 'naturally grown' and certified organic foods, and patrons have come to expect such products. These markets allow producers to receive retail prices, which is necessary for most small-scale agriculture to survive financially.

Over the last 20 years there had been many media reports on intensive farming of chickens that highlighted the reliance of producers on antibiotics and other medications to keep their stock healthy. Bernard et al. (2007) reported that concerns over the use of genetically modified (GM) feedstuff and antibiotics/hormones coupled with the lack of a natural growth environment in intensive chicken production had led to increased consumer interest in medication-free pastured poultry. This paper investigates the consumer perceptions of locally-grown pastured broilers in Kentucky's farmers' markets. Results of this paper could be useful tools to delineate the type of chicken products that farmers' market consumers prefer.

The agricultural marketing literature has many examples of farmers' market research. Govindswamy et al. (1998) provided general-

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ized attitudes, preferences, and characteristics of consumers at farmers' markets. They reported consumption trends of fresh fruits/vegetables and organic produce. Using New Jersey data, they discovered that lack of knowledge arising out of poor promotional efforts was a chief reason for consumers to not attend farmers' markets. As expected, most farmers' market consumers placed a high premium on freshness and quality of the produce and most were willing to pay a premium for supporting local agriculture.

Henneberry and Agustini (2004) reported results of a survey of consumers, producers, and managers of Oklahoma farmers' markets. Consumers reported that purchasing fresh, high quality produce and supporting local farmers were the primary reasons for shopping in farmers' markets. These consumers did not consider "price shopping" as a very important reason for shopping at farmers' markets. Producers cited "receiving retail prices" as the most common reason for using farmers' market as their outlet: however, having customer interaction also was another important reason. Farmers' market managers indicated that they never received any specialized training for their jobs at the farmers' markets. Econometric results showed that urban and suburban consumers were much more likely to patronize farmers' markets located conveniently near urban areas.

MATERIALS AND METHODS

Data for this study came from a 2005 survey of consumers in Kentucky's farmers' markets conducted in collaboration with the Kentucky Department of Agriculture (KDA). The survey was conducted by face-to-face interviews during which respondents tasted different Kentucky-grown foods and answered questions regarding 1) familiarity and willingness to purchase food products in farmers' markets, 2) visiting and spending patterns in a farmers' market, and 3) consumer demographics. A total of 166 useful observations were obtained from Ashland (N = 20), Corbin (N = 12), Erlanger (N = 10), Frankfort (N = 27), Lawrenceburg (N = 5), Louisville (N = 26), Lexington (N = 12), Owensboro (N = 23), and Paducah (N = 31) farmers' markets. These markets were chosen randomly from a subset of Kentucky farmers' markets, available to the KDA that had been identified to be strongly patronized by consumers. Because chickens were available only at the Erlanger, Louisville, Lexington, and Owensboro farmers' markets, data from these sources were used in our analyses. The relatively low number of respondents in some markets was due to the scheduling of data gathering activities during the week when the survey staff were available. Queries made to farmers' market managers indicated that the number of patrons varied based upon the season, day of the week, weather, etc. For example, the Lexington farmers' market had as few as 85 patrons per day during certain times of the year and occasionally as many as 500 patrons per day. Attendance in Owensboro and other farmers' markets was usually from (fewer than) 100 to 250 patrons per day (Table 1).

Applying the work of Lancaster (1966), which stated that consumers derived utility from attributes that goods possess instead of the goods *per se*, we describe chickens sold in farmers' markets as a collection of attributes such as the product's price, the product's form (i.e., whether it was a whole chicken or certain cuts), whether the chicken was certified organic or if it were raised in Kentucky. These attributes and their levels (Table 2) were chosen to represent chicken characteristics that are both relevant in a farmers' market setting and under control of producers/processors.

Consumer preference data indicated the importance placed on these attributes on a five-point Likert scale: very important, important, somewhat important, slightly important, and not important (Wuensch 2005). These data were used to generate binary (0/1) variables for product attributes such as price, product origin (local vs. imported), etc. Each variable was equal to 1 if consumers considered the corresponding attribute to be at least "somewhat important," and 0 if otherwise. It was hypothesized that consumer demographics might exert a systematic influence over their ascribed importance for different product attributes (Dasgupta et al. 2000). Hence, several demographic parameters (Table 1) were used as independent variables in a logistic regression model (eEquation 1) (Greene 1993). P[consumer i considers attribute j to be important] = $\Lambda(\boldsymbol{\beta}_j' \times \mathbf{X}_i)$ where Λ

Table 1. Distribution of key demographic information expressed as a percentage of respondents in each market.

	Farmers' market								
	Ash[a]	Cor[b]	Lex[c]	Lou[d]	Fra[e]	Law[f]	Pad[g]	Erl[h]	Own[i]
Age									
21 or less	0.00	0.00	0.00	0.00	0.00	0.00	6.45	0.00	0.00
21–35	5.00	8.33	16.67	30.77	11.11	0.00	12.90	0.00	13.04
36–50	15.00	41.67	25.00	19.23	40.74	60.00	32.26	20.00	17.39
51–65	50.00	16.67	58.33	30.77	25.93	40.00	41.94	40.00	21.74
66 or more	30.00	33.33	0.00	11.54	14.82	0.00	3.23	20.00	39.13
Education									
Not high school grad	10.00	8.00	0.00	7.69	0.00	0.00	3.23	0.00	4.35
High school grad	30.00	8.33	0.00	11.54	18.52	0.00	6.45	10.00	26.09
Some college	25.00	50.00	16.67	23.08	22.22	40.00	38.71	30.00	26.09
4-year degree	10.00	25.00	25.00	15.39	40.74	0.00	25.81	20.00	26.09
More advanced degree	25.00	8.33	58.33	34.62	14.82	60.00	22.58	20.00	8.70
Race									
African American	5.00	8.00	0.00	46.15	18.52	40.00	12.90	0.00	13.04
Native American	0.00	8.33	8.33	0.00	0.00	0.00	6.45	10.00	13.04
Caucasian	85.00	75.00	83.33	42.31	74.07	60.00	67.74	60.00	65.22
Asian	0.00	0	0.00	0.00	0.00	0.00	3.23	0.00	0.00
Hispanic	0.00	8.33	0.00	0.00	3.70	0.00	3.23	0.00	0.00
Other	0.00	0	0.00	0.00	0.00	0.00	3.23	0.00	0.00
Residence									
Urban	45.00	17.00	41.67	80.77	33.33	20.00	22.58	40.00	47.83
Suburban	40.00	8.33	41.67	11.54	25.93	40.00	41.94	30.00	30.45
Rural	15.00	66.67	8.33	0.00	29.63	40.00	29.03	10.00	4.35
Annual income									
$20K or less	10.00	8.00	0.00	15.39	11.11	0.00	9.68	0.00	13.04
>$20K but <$40K	20.00	8.33	16.67	26.92	7.41	0.00	22.58	20.00	26.09
>$40K but <$60K	20.00	0.25	33.33	23.08	22.22	40.00	9.68	20.00	17.39
>$60K but <$80K	20.00	33.33	8.33	11.54	29.63	20.00	22.58	0.00	8.70
>$80K but <$100K	0.00	0	8.33	3.85	14.82	0.00	9.68	20.00	0.00
$100K or more	5.00	16.67	16.67	7.69	7.41	0.00	16.13	10.00	4.35
Household size									
Mean	2.47	3.00	2.9	2.31	2.65	1.80	2.59	2.71	2.20
Standard deviation	0.96	1.34	1.52	1.01	1.27	1.3	1.12	1.70	1.11
N	20	12	12	26	27	5	31	10	23

[a] Ashland farmers' market, [b] Corbin farmers' market, [c] Lexington farmers' market, [d] Louisville farmers' market, [e] Frankfort farmers' market, [f] Lawrenceburg farmers' market, [g] Paducah farmers' market, [h] Erlanger farmers' market, [i] Owensboro farmers' market.

represents the Logistic cumulative distribution function, $\boldsymbol{\beta}_j$ represents a (k × 1) vector of regression coefficients for the jth attribute of a product, and $\mathbf{X}_i$ represents a (k × 1) vector of consumer characteristics, as discussed above.

Examples of the above methodology exist in Foltz et al. (1999) and Dasgupta et al. (2000)

Table 2. Product attributes and corresponding levels associated with chickens offered in farmers' markets that were used in the conjoint model.

Attributes:	Levels:
Price	$6.60/kg ($3.00/lb), $9.90/kg ($4.50/lb), or $13.20/kg ($6.00/lb)
Form	Whole, Leg quarters, or Breast quarters
Type	"Organic" or "Non organic"
Origin	"KY grown" or "Not KY grown"

where consumer preferences, elicited on a Likert scale, were regressed with respect to demographic parameters to characterize respondents that have systematically demonstrated a high/low preference for products. By applying Equation 1 to our data, we developed a logistic likelihood function for each product attribute, which was maximized by selecting the appropriate $\boldsymbol{\beta}_j$s. The $\boldsymbol{\beta}_j$s were used to identify subgroups of consumers that exhibited a significantly (i.e., $P \leq 5\%$) higher/lower preference for different chicken products.

The farmers' market survey also collected data for a conjoint experiment; however, only the Louisville and Owensboro farmers' market contributed useful data for this experiment. Conjoint analysis was used to evaluate buyer

acceptance of multi-attribute products (Wirth et al. 1990). Product attributes were carefully selected based upon characteristics that are under control of sellers and thought to be important to buyers. In this study, broilers sold in farmers' markets were described by the following attributes 1) price (alternative levels: \$6.60/kg or \$3.00/lb, \$9.90/kg or \$4.50/lb, \$13.20/kg or \$6.00/lb), 2) form (alternative levels: whole, leg quarter, breast quarter), 3) origin (alternative levels: Kentucky-grown, not Kentucky grown), and 4) type (alternative levels: certified organic chicken, not organic). Other attributes such as chicken breed, broiler size, etc., were considered to follow industry standards. Using the three levels of "price" and "form" attributes, and two levels of "origin" and "type" attributes, respectively, a list of $3 \times 3 \times 2 \times 2 = 36$ alternative product profiles was developed (Dasgupta et al. 2007).

If respondents rated all 36 product profiles, the resulting conjoint experiment would be a full factorial design. However, requiring respondents to rate 36 products is unreasonable; hence, an orthogonal fractional factorial design developed from the full factorial experiment was used to reduce the total number of products to be rated in the survey from 36 to 11. Orthogonal fractional factorial designs are used in conjoint analyses in which a subset of all factor-treatment combinations is selected to allow estimation of the main effects of each product attribute on consumer perceptions of the product (Dasgupta et al. 2007). Wirth et al. (1990), Halbrendt et al. (1995), and Harrison et al. (1998) provided details of methodology and justification for using orthogonal fractional-factorial designs in conjoint experiments.

The 11 product profiles (Table 3) were selected from the 36 total profiles using SAS %mktruns and %mktex macros (SAS 2008). Surveyed consumers rated each of the 11 product profiles on a 0 to 10 scale (0: extreme dislike; 10: extreme like). The 11 profiles included two "holdout" profiles which were used to validate the conjoint results by comparing predicted ratings of holdout products to their average observed ratings. Holdout products were chosen to represent likely products available in farmers' markets in the future. Hence, their presence in the conjoint experiments allowed us to investigate the consumer utility for potential future products.

A conjoint analytical model expresses a causal relationship between a buyer's utility to the combination of attribute levels that defines the product (Bernard et al. 2007). Using the preference ratings as proxy for utility, model (2) was developed to investigate which product attributes enhanced or diminished a typical respondent's utility. We adopted the part-worth utility model that provided the greatest flexibility in the shape of the utility function (Wirth and Davis 2003). This analytical model allowed for separate estimates of the effect of each level of every attribute on the mean rating/utility of a product. Mean deviation dummy variables were used in (2) (Harrison et al. 1998). The estimated coefficients (β's) are called "part worths" of attribute levels towards calculating the consumer utility. Using product attributes (Table 2) and following the conjoint methodology outlined in Harrison et al. (1998) and Dasgupta et al. (2007), the conjoint model used in this paper is outlined as (Equation 2) $R_{ij} = \beta_0 + \beta_1 \times \text{PriceMed}_i + \beta_2 \times \text{PriceHi}_i + \beta_3 \times \text{KY-Grown}_i + \beta_4 \times \text{BreastQuarter}_i + \beta_5 \times \text{LegQuarter}_i + \beta_6 \times \text{Organic}_i + \varepsilon_{ij}$, where R_{ij} represents the rating of product 'i', made by respondent 'j', where PriceMed (PriceHi) is a dummy variable which takes values of 1, 0, -1 if product price was \$9.90/kg (\$13.20/kg), \$13.20/kg (\$9.90/kg), or \$6.60/kg, respectively. KY-Grown is a dummy variable that takes a value of 1 (-1) if a chicken product werre grown (not grown) in Kentucky; BreastQuarter (LegQuarter) was a dummy variable 1, 0, or -1 if the chicken product form were a breast quarter (leg quarter), leg quarter (breast quarter), or whole-dressed, respectively. Organic is a dummy variable that takes a value of 1 (-1) is a chicken product were (were not) certified organic.

Equation (2) was estimated with 2-limit TOBIT regressions using the SAS QLIM procedure (SAS 2008).

The part worth estimates were used to calculate the relative importance (RI) of each product attribute to the respondents. The RI results help suppliers to prioritize their attention on those product attributes that their customers consider to be highly important in suppliers efforts to develop an "ideal" product for consumers. The RI of a product attribute is calculated by first measuring the

Table 3. Hypothetical chicken products rated by survey respondents in farmers' markets as part of a conjoint experiment.

Product number	Price: $/kg ($/lb)	Form	Type	Origin
1	$13.20 ($6.00)	Whole	Organic	Not KY grown
2	$9.90 ($4.50)	Whole	Non-organic	KY grown
3	$9.90 ($4.50)	Leg quarter	Organic	Not KY grown
4	$6.60 ($3.00)	Whole	Non-organic	Not KY grown
5	$13.20 ($6.00)	Breast quarter	Non-organic	Not KY grown
6	$6.60 ($3.00)	Leg quarter	Non-organic	Not KY grown
7	$6.60 ($3.00)	Breast quarter	Organic	KY grown
8	$13.20 ($6.00)	Leg quarter	Non-organic	KY grown
9	$9.90 ($4.50)	Breast quarter	Non-organic	Not KY grown
10 (Holdout)	$9.90 ($4.50)	Leg quarter	Organic	Not KY grown
11 (Holdout)	$13.20 ($6.00)	Breast quarter	Organic	KY grown

range of part worth estimates over all levels of that attribute. The RI of an attribute is expressed as the ratio of the range of part worth estimates of different levels of the attribute over the sum of such ranges for all attributes of the product, i.e., RI(Attribute i) = Part worth range(Attribute i) / Part worth range (Attribute j), where 'j' indexes all relevant attributes of the product (Halbrendt et al. 1995).

RESULTS

Surveyed consumers indicated their willingness to purchase three alternative chicken products (whole-dressed chickens, breast quarters, and leg quarters) (Table 4). By aggregating data from all surveyed farmers' markets, a weighted average of results (Table 4) indicated that overall 65%, 68%, and 88% of consumers preferred chicken leg quarters, whole dressed chickens, and chicken breast quarters, respectively. Chicken breast quarters and chicken leg quarters were the most and least popular products, respectively.

Respondents were asked to choose product attributes such as product form, type, size and price to formulate their "ideal" chicken product. The most popular product was certified organic chicken breast quarters packaged in 0.83 kg (2 lb) portions and priced between $2.20/kg and $4.40/kg ($1/lb and $2/lb) (Table 5). Also 34% of respondents preferred whole-dressed chickens over other, more processed, product types. The "$6.60/kg ($3/lb)–$8.80/kg ($4/lb)" price category was the second most popular of all price ranges available to the respondents.

Logit regression results indicated that a consumer's demographics did impact their opinions about the price of chickens. A respondent's age and the distance of residence from the farmers' market made them significantly more sensitive to chicken prices (Table 6). Older respondents and those living farther from a farmers' market considered price to be an important aspect in making purchasing decisions than other demographic groups of respondents. A contingency table analysis found that a respondent's race significantly affected their opinions: Caucasian respondents were more sensitive to having "certified organic" chickens; they considered this attribute to be significantly more important in making purchasing deci-

Table 4. Percentage of respondents from each farmers' market that would purchase the indicated product in a farmers' market, retail grocery store, or directly from farmers.

Product	Farmers' market								
	Ash[a]	Cor[b]	Lex[c]	Lou[d]	Fra[e]	Law[f]	Pad[g]	Erl[h]	Own[i]
Whole-dressed chicken	61	67	75	65	85	20	53	78	79
Chicken breast quarters	95	100	100	84	89	100	71	89	95
Chicken leg quarters	56	70	91	50	74	20	90	28	55

[a] Ashland farmers' market, [b] Corbin farmers' market, [c] Lexington farmers' market, [d] Louisville farmers' market, [e] Frankfort farmers' market, [f] Lawrenceburg farmers' market, [g] Paducah farmers' market, [h] Erlanger farmers' market, [i] Owensboro farmers' market.

Table 5. Ideal chicken product as indicated by respondents. Data aggregated over Lexington, Louisville, Erlanger, and Owensboro farmers' markets.

	Frequency (percentage)
Product form	
Whole chicken	19 (35)
Leg quarter	4 (7)
Breast quarter	32 (58)
Product type	
Certified organic	34 (67)
Not certified organic	17 (33)
Package size	
0.41 kg (1 lb)	3 (5)
0.83 kg (2 lb)	33 (62)
2.07 kg (5 lb)	13 (25)
>2.07 kg (5 lb)	4 (8)
Price in $/kg ($/lb)	
<$2.20/kg ($1/lb)	3 (10)
≥$2.20/kg ($1/lb) and <$4.40/kg ($2/lb)	8 (28)
≥$4.40/kg ($2/lb) and <$6.60/kg ($3/lb)	3 (10)
≥$6.60/kg ($3/lb) and <$8.80/kg ($4/lb)	7 (24)
≥$8.80/kg ($4/lb) and <$11.00/kg ($5/lb)	5 (17)
≥$11.00/kg ($5/lb) and <$13.20/kg ($6/lb)	2 (7)
≥$13.20/kg ($6/lb) and <$15.40/kg ($7/lb)	0 (0)
≥$15.40/kg ($7/lb) and <$17.60/kg ($8/lb)	1 (4)
≥$8	0 (0)

sions than non-Caucasian respondents (chi-squared test statistic = 4.13; P = 0.04; Mantel-Haenszel chi-squared test statistic = 4.06; P = 0.04; N = 53).

Conjoint regression results (Table 7) showed that a consumer's rating of a chicken product was significantly reduced when price was $13.20/kg ($6/lb) and the product form was "leg quarters." However, the ratings significantly improved if product form was "breast quarters" (except at Owensboro farmers' market), or the chickens were locally grown, or were certified organic animals.

Two holdout products were investigated to evaluate the consumer preferences for chicken products that can be feasibly offered in farmers' markets. These holdout products were "organic chicken leg quarters, not KY grown, priced at $9.90/kg ($4.50/lb)" and "organic chicken breast quarters, grown in KY, priced at $13.20/kg ($6.00/lb)," and their average respondent ratings were 2.60 and 3.44, respectively. Using the conjoint results for the pooled data, the predicted ratings for holdout products 1 and 2 were 2.70 and 4.48, respectively. These results clearly show that offering the preferred form (i.e., breast quarters) allowed products to command a higher price and simultaneously provided buyers with a greater utility, as captured by the product ratings.

Using the conjoint results, the relative importance of different chicken attributes was calculated (Table 8). A product's price was deemed to be the most important attribute. This was followed by a product's form (i.e., whole chicken, leg quarters, or breast quarters), type (i.e., organic or not organic), and origin (i.e., locally grown or not) for the Louisville farmers' market. In the Owensboro farmers' market, a product's form, origin, and type were considered by the respondents to be in order of decreasing relative importance.

CONCLUSIONS

Our study of consumer perceptions of chickens sold in Kentucky's farmers' markets revealed that the majority of respondents were willing to purchase locally-grown chickens. Our results showed that chicken breast quarters were the most preferred product

Table 6. Results of a logistic regression on the importance that consumers place on chicken price in farmers' markets to identify systematic effects of demographic parameters[a].

	Regressors[b]						
	Intercept	Age > 36	White	Hi-income	Distance	College	VisitOften
Coefficient estimate	−0.73	1.62	0.07	−1.05	0.38	−0.91	0.54
Standard error	1.70	0.84	0.74	0.99	0.21	1.24	0.85
P-value	0.67	0.05	0.92	0.29	0.07	0.46	0.53

N = 53; Generalized R^2 = 0.21; LR test = 11.35 (P = 0.07); Tau-a = 0.26.

[a] Dependent variable: Price Important = 1 if respondents consider product price to be very important, important or somewhat important on a Likert scale; Price Important = 0 if respondents consider price to be slightly important or not important.

[b] Age > 36 is a dichotomous variable which is '1' if respondent's age exceeds 36; '0' otherwise. Hi-income is a dichotomous variable which is '1' if respondents age exceeds 36; '0' otherwise. Distance in miles from respondent's residence to the farmers' market. College is a dichotomous variable which is '1' if respondent has college education; '0' otherwise. VisitOften = '1' if respondent visits a farmer' market at least weekly; otherwise it is '0'.

Table 7. TOBIT regression results for estimating conjoint model for data from Louisville and Owensboro farmers' markets. Dependent variable: product ratings on a 0–10 scale.

Regressors	Farmers' market		
	Louisville	Owensboro	Pooled data
Intercept	2.69[a] (<0.0001[b])	3.05 (<0.0001[b])	2.88 (<0.0001[b])
PriceMed	−0.38 (0.440)	0.32 (0.430)	−0.02 (0.961)
PriceHi	−1.73 (0.001[b])	−1.27 (0.002[b])	−1.50 (<0.0001[b])
KY grown	0.93 (0.013[b])	0.68 (0.026[b])	0.80 (0.001[b])
Breast Quarter	1.33 (0.007[b])	0.63 (0.124)	0.97 (0.003[b])
Leg Quarter	−1.00 (0.045[b])	−1.05 (0.011[b])	−1.03 (0.001[b])
Organic	1.11 (0.003[b])	0.51 (0.09[c])	0.81 (0.001[b])
N	171	162	333
Likelihood Ratio Index	0.057	0.033	0.043

[a] Coefficient estimate (P-value).
[b] The estimated coefficient is significantly different from zero for $\alpha = 5\%$.
[c] The estimated coefficient is significantly different from zero for $\alpha = 10\%$.

form and whole-dressed chickens were preferred over leg quarters. This is encouraging for producers/processors wanting to sell whole-dressed chickens, and it shows that the commonly-held notion that highly processed food products command greater consumer demand than less-processed versions of the same food may not always be true.

The main conclusions from this study are 1) younger consumers and those that live close to farmers' markets were less price sensitive than other consumers; 2) Caucasians were more willing to buy certified organic chickens than consumers of other racial groups; and 3) the product form of chickens (i.e., whole chickens vs. different cuts) was more important to buyers than the knowledge of whether the chickens were certified organic or guaranteed to be produced in Kentucky, although the latter two attributes received 20% RI, each.

The "ideal" chicken product for farmers' markets patrons was chicken breasts quarters that were certified organic, available in 0.83 kg (2 lb) packages, and priced from $2.20–$4.40/kg ($1–$2/lb). While this price range is likely to be unacceptable for producers/processors for breast quarters, it is more likely that such prices are feasible for whole-dressed chickens, which product form had received 68% of respondent approval, on average. Hence, this study's recommendations are for continued investigations into the willingness-to-pay for Kentucky-grown whole-dressed chickens among farmers' markets patrons.

Table 8. Relative importance of different chicken product attributes.

Attributes	Farmers' market		
	Louisville	Owensboro	Pooled data
Price	37.45%	35.37%	36.66%
Form	22.76%	26.67%	24.25%
Type	21.68%	16.37%	19.64%
Origin	18.11%	21.59%	19.45%

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Habitat Characteristics Associated with American Woodcock (*Scolopax minor* Gmelin) Nests in Central Kentucky

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ABSTRACT

Fifteen American woodcock (*Scolopax minor*) nests were located in central Kentucky between February 1998 and April 1999. Twelve habitat variables were measured within a 0.04 ha circular plot centered at each nest site and at randomly located no-nest (control) sites. Mean angular canopy cover at the nest, and mean number of shrubs and trees in the 2.5–15.2 cm DBH range within the nest plot were found to be significantly different ($P < 0.05$) from control sites. The habitat variables of note associated with woodcock nest sites in Kentucky mirror the general trend reported elsewhere, i.e., the importance of mid-story and horizontal plant cover.

KEY WORDS: Kentucky, American woodcock, *Scolopax minor*, nesting

INTRODUCTION

Woodcock (*Scolopax minor* Gmelin) populations have experienced declines over the last decades that appear to be due to land use changes (Dobell 1977). Owen et al. (1977) and Dwyer et al. (1983) reported woodcock habitat in most states was declining because of successional trends in vegetation, urbanization, and commercial development. Although habitat used by woodcock for brood rearing and nesting has been studied throughout the breeding range (Shelton 1967; Liscinsky 1972; Gregg and Hale 1977; Roboski and Causey 1981; Coon et al. 1982; Dwyer et al. 1982; Parris 1986; Kinsley and Storm 1989; McAuley et al. 1996), the results are contradictory (Sepik et al. 1989).

Woodcock nests in Kentucky have been found associated with a source of water and weeds or brush (Russell 1959) and brush/thicket habitat (Abel and Ritchison 1999). In the American Woodcock Management Plan, the U.S. Fish and Wildlife Service (USFWS 1997) noted that additional research was needed to determine habitat requirements in southern areas of the woodcock's range. Kentucky is regarded as a stopover and nesting area for woodcock (USFWS 1997). The objective of this study was to enhance the data base relative to vegetation characteristics associated with American woodcock nesting habitat in central Kentucky.

MATERIALS AND METHODS

The study was conducted from February 1998 to April 1999 on the Blue Grass Army Depot (BGAD) and Central Kentucky Wildlife Management Area (CKWMA) in Madison County, and on the Clay Wildlife Management Area (CWMA) in Nicholas County, KY. The BGAD is composed of open fields, woodlots, and areas in various stages of successional growth (Thomas 1994). Sparks (1990) described the vegetation on the CKWMA as a fragmented mosaic consisting of wooded areas, old fields, and agricultural fields. The CWMA consists of upland hardwoods, croplands, old fields, and bottomland hardwoods associated with the Licking River (Krantz 1985). Additional study area details are provided in Harris (2000).

American woodcock nests were located using trained bird dogs (Sheldon 1967). By using a nest as the center of a 0.04 ha circular plot; the following parameters were assessed: (1) light intensity (Coon et al. 1982), (2) distance from the nest to nearest edge (Coon et al. 1982), (3) linear amount of edge in plot,

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Table 1. Habitat parameters associated with American woodcock nest sites (N = 15) and control (no-nest) sites in central Kentucky. Data are mean ± SD, with range in parentheses.

Variable	Nest	Control
Light intensity	37,754.6 ± 32,950.8 luxes (510–93,100)	24,349.3 ± 24,748.5 (1520–73,600)
Distance from nest to nearest edge	9.0 ± 7.9 m (0–25)	18.6 ± 18.2 (0–45)
Linear amount of edge in plot	18.8 ± 13.5 m (0–48)	13.3 ± 15.4 (0–42)
Distance to nearest human disturbance	65.8 ± 141.5 m (1–500)	37.3 ± 74.5 (0–300)
Mean height of all trees in plot	10.2 ± 4.6 m (3.4–17.4)	6.4 ± 6.3 (0–15.7)
Angular canopy cover at nest	31.4 ± 18.2% (0–61)	17.6 ± 21.2 (0–69)
Number of shrubs within plot	19.2 ± 16.7 (4–59)	12.6 ± 12.2 (0–36)
Number of trees within plot.	45.0 ± 29.4 (0–106)	20.8 ± 25.8 (0–84)
Basal area of trees within plot	13.1 ± 7.1 m^2/ha (0–27.5)	6.9 ± 7.8 (0–21.5)
Number of trees <2.5 cm dbh	2.3 ± 4.8 (0–15)	0.4 ± 1.1 (0–4)
Number of trees 2.5–15.2 cm dbh	28.6 ± 14.4 (6–63)	14.9 ± 12.5 (0–37)
Number of trees >15.2 cm dbh	17.6 ± 22.1 (0–81)	9.2 ± 16.2 (0–62)

(4) distance from nest to nearest human disturbance, (5) mean height of all trees in the plot (erect woody vegetation ≥6 m in height), (6) angular canopy cover directly over nest, determined with a spherical densitometer (Nuttle 1997), (7) number of shrubs (erect woody vegetation >2 m and <6 m in height) within the plot, (8) total number of trees within the plot, (9) basal area of trees, determined using a wedge prism (BAF = 10; Avery 1969), and (10) number of trees in each of the following categories: <2.5 cm dbh, 2.5–15.2 cm dbh, >15.2 cm DBH.

The horizontal structure of vegetation, in conjunction with vertical structure, has been reported to influence avian habitat preference (Rotenberry and Wiens 1980). In this study, horizontal cover was defined as the amount of obstruction to the horizontal view through the vegetation, i.e., the number of stems in the shrub layer, and was equated to the number of shrubs within the plot.

Each nest site was paired with a random no-nest (control) site for statistical analysis. To determine the location of a control site, a two-digit and three-digit random number were selected. The two digit number indicated the number of paces walked away from the actual nest and the three digit number designated the direction (compass heading) in which to walk. Because post-hoc analysis revealed 4 of the 12 habitat variables measured were not normally distributed (Shapiro-Wilk statistic, SAS 1989), the non-parametric Sign Test (McClave et al. 1997) was used to compare nest sites with control sites. The Bonferroni Method (Sokal and Rohlf 1995) was employed to adjust the alpha level used to reject individual hypotheses so that the experiment-wise error rate was maintained at 0.05 over the entire series of individual tests.

RESULTS AND DISCUSSION

Fifteen American woodcock nests were located during the study (6, 5, and 4 nests were located on the BGAD, CKWMA, and CWMA study sites, respectively). Mean values for angular canopy cover at the nest (Sign Test, df = 1,14, $P = 0.007$), and number of shrubs ($P = 0.035$), trees ($P = 0.003$), and trees in the 2.5–15.2 cm dbh range ($P = 0.001$) were all higher in the nest plots compared with the control plots (Table 1).

Ground nesting birds were postulated to preferentially select nest sites in dense shrubs or undergrowth for nest concealment: (1) to decrease predator detection because of the site's proximity to foraging areas or (2) because the site contained a favorable microclimate (Holway 1991). Nest predation is one of the most significant factors contributing to unsuccessful nests (Kilgo et al. 1996). Thus, nest concealment becomes of paramount importance to the reproductive success of a ground nesting bird such as the American woodcock.

Lennington (1980) noted that for some species of birds, the proximity to food sources is the principal criterion of nest site selection. This has been postulated as having an influence on woodcock nest site selection because of the bird's dependence on earth-

worms and the moist soils with which the worms are associated (Sheldon 1967). Selecting nesting locations in areas of dense horizontal cover (e.g., dense understory and shrub layers) would result in nest sites being associated with more favorable temperature regimes (i.e., microclimates, Holway 1991). Each of the factors noted in this discussion have been indicated as influencing woodcock nest site selection in various portions of the bird's range (Gregg and Hale 1977; Roboski and Causey 1981; Coon et al. 1982; McAuley et al. 1996; Abel and Ritchison 1999).

Woodcock in this study appeared to have been choosing locations that enhanced the vertical canopy and horizontal shrub cover associated with the nest site; features noted of importance in nesting studies throughout the bird's range (Coon et al. 1982; McAuley et al. 1996). The vertical cover exemplified in this study consisted of tree limbs and branches, because all leaf foliage was basically absent during the nesting season. Attributes of horizontal cover of note were number of stems in the shrub layer and number of relatively small diameter (2.5–15.2cm DBH) trees in the vicinity of the nest. All of the obstructions could decrease the attractiveness of the nest site as hunting areas for avian and terrestrial predators, thereby enhancing the nesting success and recruitment of woodcock.

The mid-successional plant community characteristic of woodcock nest sites examined in this study represent habitat that may be developed through various management procedures, e.g., timber harvest, burning (Krementz and Jackson 1999). Conversely, there are habitat-modifying practices, e.g., continuous cattle grazing, brush hogging or mowing that when employed in certain areas may be detrimental to the establishment or maintenance of woodcock nesting habitat.

The development or preservation of plant communities exemplifying the nesting habitat features of importance identified in this study would create habitat of value to nesting woodcock. As observed in this study, when established in juxtaposition to riparian or wetland vegetation, the resulting plant community would create nesting habitat in close proximity to optimal feeding habitat. Nest sites located near areas containing high densities of earthworms provide optimal foraging for the chicks and promote brood success (Sheldon 1967).

The number of nests examined in this study was relatively small, a reoccurring problem in the study of nesting American woodcock (Gregg and Hale 1977; Roboski and Causey 1981; Coon et al. 1982). Despite the small sample size, the habitat variables associated with woodcock nest sites in Kentucky mirror the general trend reported in the literature, i.e., the importance of mid-story and horizontal plant cover.

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Analysis of Modulation of Foxp3 Expression in $CD4^+CD25^+$ Regulatory Cells from NOD Mice

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ABSTRACT

$CD4^+CD25^+$ regulatory cells control the development of autoimmunity, including type 1 diabetes, and the transcription factor Foxp3 is crucial for the development and function of these cells. The decreased effectiveness of regulatory T cell function in diabetes-prone NOD mice is due to the failure of NOD APC to activate regulatory T cells properly. In the present study, we examined the parameters that modulate Foxp3 expression in $CD4^+CD25^+$ regulatory cells. We found that $CD4^+CD25^+$ cells from congenic diabetes-resistant NOD mice exhibited intermediate levels of Foxp3 compared with regulatory cells from B6 mice and Sick NOD mice. Using an *in vitro* Foxp3 induction system, we found that APC from diabetes-resistant congenic NOD mice also exhibited an intermediate ability to maintain expression of Foxp3 in $CD4^+CD25^+$ cells. Finally, NOD APC pre-treated with a microbial component, lipoteichoic acid, regained their ability to maintain Foxp3 expression in $CD4^+CD25^+$ cells *in vitro*. APC from CFA-treated NOD mice that were transferred into recipient NOD mice could induce Foxp3 expression in $CD4^+CD25^+$ cells *in vivo*. Altogether, these data suggest that Foxp3 expression in $CD4^+CD25^+$ cells from NOD mice can be modulated by optimal stimulation by APC.

KEY WORDS: Type 1 diabetes, regulatory cells, Foxp3 expression, antigen presenting cells, *Idd*

INTRODUCTION

Non obese diabetic (NOD) mice develop spontaneous diabetes that resembles human Type I diabetes. The disease incidence varies from 60 to 90% in female NOD mice depending on the animal facility and is much lower in males. NOD mice appear to have a dysregulated immune response, including deficiency in two regulatory cell populations, NKT and $CD4^+CD25^+$ regulatory T cells (Gombert 1996; Wu 2002) and antigen-presenting cell (APC) function (Serreze 1993; Piganelli 1998; Dahlen 2000; Lee 2000). $CD4^+CD25^+$ regulatory T cells play a crucial role in controlling autoimmune disease development, including type 1 diabetes. Although controversial, the percentage and function of these cells have been found to be altered in NOD mice (Salomon 2000; Kishimoto 2001; Wu 2002; Alard 2006) and diabetic patients (Kukreja 2002). We recently have shown that the defect in regulation observed in NOD mice appears to lie in the inability of NOD APC to activate $CD4^+CD25^+$ regulatory T cells (Alard 2006). Moreover, we have compelling data showing that complete Freund's adjuvant (CFA) injection into NOD mice restores functional APC and regulatory cells and prevents diabetes development (Manirarora 2008).

$CD4^+CD25^+$ regulatory T cells account for 5–10% of $CD4^+$ cells in healthy mice and humans and are crucial for controlling the development of autoimmune diseases. Although the mechanisms of action of $CD4^+CD25^+$ regulatory T cells are still controversial (Sakaguchi 2004), these cells absolutely require activation (Takahashi 1998; Thornton 1998), presumably by APC, to mediate regulation. Recently, a transcription factor, Foxp3, has been found to be critical for $CD4^+CD25^+$ regulatory T cell development and function. $CD4^+CD25^+$ regulatory T cells from Foxp3-deficient mice are not functional, and naive T cells forced to express Foxp3 exhibit regulatory function (Fontenot 2003; Hori 2003; Khattri 2003). Few studies have confirmed the relationship between Foxp3 and regulatory cell function using transgenic mice expressing GFP under the control of the Foxp3 promoter (Sakaguchi 2004; Fontenot 2005). More importantly, a recent study has found a correlation between levels of Foxp3 expression and regulatory function (Wan 2007) suggesting that failure to maintain optimal Foxp3 expression could compromise regulatory cell function and lead to autoimmune disease development.

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$CD4^+CD25^+$ regulatory cells from NOD mice were found to express diminished level of Foxp3 at the mRNA level (Pop 2005). We have confirmed this observation at the protein level and have shown that NOD APC were less efficient at maintaining Foxp3 expression in $CD4^+CD25^+$ regulatory T cells from NOD mice (Manirarora 2008). In the current study, we have investigated in more detail the defect in Foxp3 expression in $CD4^+CD25^+$ regulatory T cells from NOD mice. We have examined whether the levels of Foxp3 expressed by $CD4^+CD25^+$ regulatory T cells was normal in diabetes-resistant congenic NOD mice. We also have examined whether APC from diabetes-resistant congenic NOD mice were able to maintain Foxp3 expression in $CD4^+CD25^+$ regulatory T cells from NOD mice. Finally we assessed whether pre-activation of NOD APC with microbial components could restore normal levels of Foxp3 in NOD $CD4^+CD25^+$ regulatory T cells *in vitro* and *in vivo*.

MATERIALS AND METHODS

Mice

Eight-fifteen week-old C57BL/6 and NOD female mice (Jackson Laboratory, Bar Harbor, ME) and congenic *Idd*3/5 and *Idd*9 NOD female mice (Taconic, Hudson, NY) were maintained under specific pathogen-free conditions as described in the Institutional Animal Care and Use Committee guidelines.

Antibodies and Flow Cytometry

APC-anti-CD25, PerCP-anti-CD4 antibodies were purchased (BD Pharmingen, San Diego, CA). One million cells were incubated with Fc block and labeled with antibodies for 20 min in DPBS 1% FCS, 0.1% $NaNO_3$, and washed twice. For Foxp3, cells were intracellularly labeled with PE-anti-Foxp3 antibodies (eBioscience, San Diego, CA) according to the manufacturer's instructions. Cells were analyzed by FACS® using a FACScalibur (Becton Dickinson, Palo Alto, CA).

T cell Depletion of Spleen Cells

Spleen cells were incubated in lysis buffer (RPMI 1640 with 1M Hepes and 0.3 g BSA) containing anti-mouse CD90 antibody (Cedarlane, Hornby, Ontario, Canada), then with Low-Tox-M rabbit complement (1:10; Cedarlane, Hornby, Ontario, Canada). The purity of the $CD3^-$ cells was consistently >95%. In some case, $CD3^-$ cells were stimulated overnight in the presence of 10 μg/ml of lipoteichoic acid (LTA),

Cell Culture for Evaluation of Foxp3 Maintenance

$CD4^+CD25^+$ regulatory T cells from three mice were pooled and sorted to >95% purity (MoFlo®, DakoCytomation, Fort Collins, CO) and cultured in multiple wells ($2–4 \times 10^4$ cells/well) in complete media (RPMI 1640, 10% heat-inactivated FCS, 2 mM glutamine, 10 mM HEPES, 100 U/ml pen G sodium, 100 μg/ml strep sulfate, and 1×10^{-5} 2 ME) for 18 hrs with irradiated T cell-depleted spleen cells (APC; 1×10^5) pooled from three mice and anti-CD3 antibody (0.5 μg/ml).

Local Adoptive Transfer to Evaluate Foxp3 Maintenance *in vivo*

Complete Freund's adjuvant (CFA) was prepared using non-viable desiccated *Mycobacterium tuberculosis* (H37 RA, Difco Laboratories, Detroit, MI) at 1 mg/ml in PBS and emulsified in incomplete Freund's Adjuvant (IFA; Sigma-Aldrich, St Louis, MO). Eight week-old NOD mice were injected subcutaneously with 100 μl of PBS or complete Freund's adjuvant (CFA). Three weeks later, spleen cells were harvested, depleted of T cells, and 1×10^6 cells injected subcutaneously in the footpad of 6–8 week-old NOD mice. After four days, popliteal LN were collected, and cells were labeled for Foxp3.

Statistical Analysis

Data were analyzed using the student's *t*-test. Each experiment was repeated with reproducible results at least 2 times. One representative experiment is shown in each figure or table.

RESULTS

Comparison of Foxp3 Expression in $CD4^+CD25^+$ Regulatory Cells from Diabetes-Resistant Congenic NOD Mice and Diabetes-Prone NOD Mice

Multiple genetic loci control disease susceptibility in NOD mice, and one of these loci include the MHC class II I-$A\beta^{g7}$ allele. Furthermore, congenic NOD mice bearing

Table 1. $CD4^+CD25^+$ regulatory cells from congenic diabetes-resistant mice exhibit intermediate levels of Foxp3 by comparison with cells from B6 and Sick NOD mice.

$CD4^+CD25^+$ cells	% $Foxp3^{+a}$	Foxp3 MFI[a]
B6	88	310
$B6^{g7}$	93	230
*Idd*3/5	94	220
*Idd*9	93	223
Sick NOD	87	175

[a] Splenic cells were analyzed for Foxp3 expression by FACS® after gating on $CD4^+CD25^+$ cell and percent of $Foxp3^+$ cells and mean fluorescent intensity (MFI) for Foxp3 are represented. Representative results from one of two experiments are shown.

one or more loci termed insulin-dependent diabetes (*Idd*) loci that are linked to diabetes resistance or susceptibility, such as *Idd*3 (Lyons 2000), *Idd*5 (Kissler 2006) or *Idd*9 (Lyons 2000) are highly protected from the occurrence of type-1 diabetes (Maier 2005; Hamilton-Williams 2007). Because we previously had found that $CD4^+CD25^+$ regulatory cells from NOD mice exhibited a lower Foxp3 expression than cells from B6 mice (Manirarora 2008), we first examined whether $CD4^+CD25^+$ regulatory cells from diabetic-resistant *Idd*3/5 and *Idd*9 congenic NOD mice exhibited normal levels of Foxp3. Cells from spleens of four mice were pooled and labeled with anti-CD25, anti-CD4 and anti-Foxp3 antibodies and analyzed by FACS. We found that $CD4^+CD25^+$ regulatory cells from congenic diabetes-resistant mice exhibit intermediate levels of Foxp3 compared with cells from B6 (normal control) and NOD mice (Table 1) Furthermore, $CD4^+CD25^+$ regulatory cells from $B6^{g7}$ mice that express the MHC class II I-$A\beta^{g7}$ allele of NOD mice also exhibited intermediate Foxp3 expression, suggesting that the allele I-$A\beta^{g7}$ may be involved in the intermediate Foxp3 expression observed in $CD4^+CD25^+$ regulatory cells from congenic diabetes-resistant mice.

Comparison of the Ability of APC from Diabetes-Resistant Congenic NOD Mice and Diabetes-Prone NOD Mice to Maintain Foxp3 Expression *in vitro*

We had shown in previous studies that APC from NOD mice failed to optimally activate $CD4^+CD25^+$ regulatory cells *in vitro*, as shown by decreased suppression of effector cell proliferation (Alard 2006) and lower maintenance of Foxp3 expression *in vitro* (Manirarora 2008). In the current study, we first tested whether $CD4^+CD25^+$ regulatory cells from eight week-old pre-diabetic or 12–15 week-old Sick NOD mice responded differently to APC stimulation for the maintenance of Foxp3 expression using an *in vitro* assay that we have developed (Manirarora 2008). Indeed, $CD4^+CD25^+$ regulatory cells from Sick NOD mice exhibited lower levels of Foxp3 after stimulation *in vitro* with B6, pre-diabetic or Sick NOD APC (Table 2, rows 1–3) compared with $CD4^+CD25^+$ regulatory cells from pre-diabetic NOD mice (Table 2, rows 4–6). Similarly, APC from pre-diabetic NOD mice were more efficient than APC from Sick NOD mice at maintaining Foxp3 expression in $CD4^+CD25^+$ regulatory cells from pre-diabetic (Table 2, rows 5&6) or Sick (Table 2, rows 2&3) NOD mice.

We next tested whether APC from diabetes-resistant mice could sustain normal levels of Foxp3 *in vitro*. Spleens were harvested from age-matched B6, $B6^{g7}$, *Idd*3/5, *Idd*9 and sick NOD mice. Cells were pooled from each group and depleted of T cells and cultured overnight with sorted $CD4^+CD25^+$ regulatory cells in the presence of anti-CD3 antibody. At the end of the culture, cells were labeled with anti-CD25, anti-CD4 and anti-Foxp3 antibodies and analyzed by flow cytometry. We found that APC from congenic diabetes-resistant mice had an intermediate ability to sustain Foxp3 expression *in vitro* compared with APC from either B6 or sick NOD mice (Table 3). Furthermore, APC from $B6^{g7}$ mice also were less efficient at maintaining Foxp3 expression *in vitro*. The intermediate expression of Foxp3 found in these strains of mice (Table 1) appeared to correlate with the intermediate ability of APC from these mice to maintain Foxp3 expression in $CD4^+CD25^+$ regulatory cells.

CEffects of *in vitro* and *in vivo* Activation of APC on Induction of Foxp3 Expression in $CD4^+CD25^+$ Regulatory Cells

Multiple studies have reported that Complete Freund's adjuvant (CFA), which contains *Mycobacterium tuberculosis*, can prevent diabetes development when injected into young NOD mice (McInerney 1991; Qin

Table 2. Comparison of APC and $CD4^+CD25^+$ regulatory cells from pre-diabetic NOD and Sick NOD mice for the maintenance of Foxp3 expression *in vitro* upon overnight culture of $CD4^+CD25^+$ T cells with APC and anti-CD3 antibody.

APC[a]	$CD4^+CD25^+$ cells	% Foxp3[+b]	Foxp3 MFI[b]
B6	Sick NOD	90	259
Pre-diabetic NOD	Sick NOD	98	181
Sick NOD	Sick NOD	94	146
B6	Pre-diabetic NOD	94	281
Pre-diabetic NOD	Pre-diabetic NOD	98	227
Sick NOD	Pre-diabetic NOD	88	171

[a] Irradiated T-cell depleted spleen cells.

[b] Cells pooled from multiple wells were analyzed for Foxp3 expression by FACS® after gating on CD4+CD25+ cell and percent of Foxp3+ cells and mean fluorescent intensity (MFI) for Foxp3 are represented. Representative results from one of two experiments are shown.

1993; Lee 2004). We found previously that $CD4^+CD25^+$ regulatory cells from CFA-treated NOD mice express normal levels of Foxp3, and APC from those mice recover their ability to stimulate regulatory cell function and maintenance of Foxp3 expression *in vitro* (Manirarora 2008). In the current study, we first tested whether exposure of APC to components of microorganisms *in vitro* also could render NOD APC capable of sustaining Foxp3 expression in $CD4^+CD25^+$ regulatory cells. Because CFA mediates its effect on APC via TLR2 (Lim 2002), we used a TLR2 ligand, lipoteichoic acid (LTA), isolated from *Staphylococcus aureus*. Spleens were harvested from sick NOD or B6 mice, and cells were depleted of T-cells and either left unstimulated or stimulated overnight with LTA. On the following day, cells were irradiated, washed, and used as APC in overnight cultures with $CD4^+CD25^+$ regulatory cells purified from either B6 or sick NOD mice. Cells were then labeled with anti-CD4, anti-CD25, and anti-Foxp3 antibodies and analyzed by flow cytometry. LTA-stimulated APC from sick NOD mice sustained Foxp3 expression *in vitro* compared with unstimulated APC from sick NOD mice (Table 4} Taken together, these data suggest that APC stimulated with LTA recover their ability to stimulate $CD4^+CD25^+$ regulatory cells *in vitro*.

Table 3. APC from diabetes-resistant congenic mice sustain intermediate Foxp3 expression *in vitro* upon overnight culture of $CD4^+CD25^+$ T cells with APC and anti-CD3 antibody.

APC[a]	$CD4^+CD25^+$ cells	% Foxp3[+b]	Foxp3 MFI[b]
B6	Sick NOD	94	234
B6[g7]	Sick NOD	97	206
*Idd*3/5	Sick NOD	96	180
*Idd*9	Sick NOD	94	190
Sick NOD	Sick NOD	98	167

[a] Irradiated T-cell depleted spleen cells.

[b] Cells pooled from multiple wells were analyzed for Foxp3 expression by FACS® after gating on CD4+CD25+ cell and percent of Foxp3+ cells and mean fluorescent intensity (MFI) for Foxp3 are represented. Representative results from one of two experiments are shown.

We next tested whether APC collected from CFA-treated mice could enhance Foxp3 expression *in vivo*. Spleen cells were harvested from CFA-treated or PBS-treated NOD mice, depleted of T cells, and 1×10^6 cells injected in the left footpad of 8 week-old NOD mice. Four days later, the left popliteal LN were collected, and cells labeled with anti-CD25, anti-CD4, and anti-Foxp3 antibodies and analyzed by flow cytometry. Foxp3 expression was significantly enhanced in $CD4^+CD25^+$ cells harvested from the LN of mice that had received APC from CFA-treated NOD mice compared to $CD4^+CD25^+$ cells harvested from the LN of mice that had received APC from PBS-treated NOD mice (Figure 1). These data suggest that APC activated *in vivo* with CFA and injected into recipient mice were able to enhance locally expression of Foxp3 in regulatory cells.

DISCUSSION

Foxp3 appears to be a critical transcription factor for $CD4^+CD25^+$ regulatory T cell development and function (Fontenot 2003; Hori 2003; Khattri 2003). Furthermore, regulatory function correlates with levels of Foxp3 expression (Wan 2007), suggesting that failure to maintain optimal Foxp3 expression could compromise regulatory cell function and lead to autoimmune disease development. Because $CD4^+CD25^+$ regulatory cells from NOD mice were found to express diminished

Table 4. Lipoteichoic acid (LTA) restores the ability of NOD APC to induce Foxp3 expression in $CD4^+CD25^+$ cells upon overnight culture of $CD4^+CD25^+$ T cells with APC and anti-CD3 antibody.

APC[a]	$CD4^+CD25^+$ cells	% $Foxp3^{+b}$	Foxp3 MFI[b]
B6	Sick NOD	82	177
Sick NOD	Sick NOD	86	134
LTA-B6	Sick NOD	85	212
LTA-Sick NOD	Sick NOD	87	189

[a] Irradiated T-cell depleted spleen cells.

[b] Cells pooled from multiple wells were analyzed for Foxp3 expression by FACS® after gating on CD4+CD25+ cell and percent of Foxp3+ cells and mean fluorescent intensity (MFI) for Foxp3 are represented. Representative results from one of two experiments are shown.

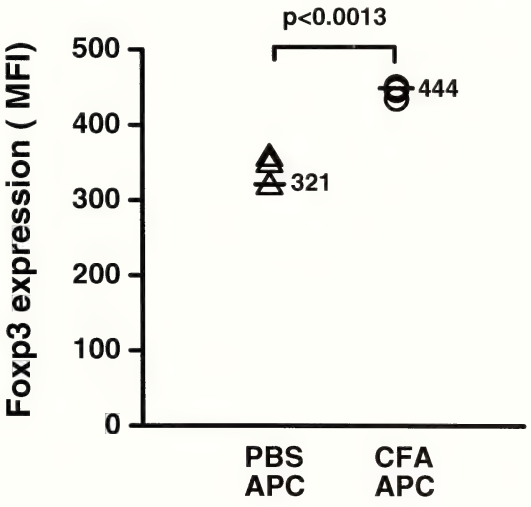

Figure 1. Induction of Foxp3 expression in $CD4^+CD25^+$ cells upon subcutaneous injection of CFA-stimulated APC. Popliteal LN cells from mice injected with PBS or CFA-stimulated APC were harvested, labeled with anti-CD4, CD25 and Foxp3 antibodies, and analyzed by FACS® after gating on CD4+CD25+ cells. Mean fluorescent intensity (MFI) for Foxp3 are represented.

levels of Foxp3 at the mRNA (Pop 2005) and protein level (Manirarora 2008), in the current manuscript we analyzed the modulation of expression of Foxp3 in $CD4^+CD25^+$ T cells from diabetes-prone NOD and diabetes-resistant congenic NOD mice. We showed that expression of Foxp3 was affected by three parameters, the expression of MHC class II I-$A\beta^{g7}$ allele by APC, the disease state, and optimal stimulation by APC.

The expression of Foxp3 in $CD4^+CD25^+$ T cells from diabetes-resistant congenic NOD mice that express the MHC class II I-$A\beta^{g7}$ allele was lower than that from B6 mice but higher than that of NOD mice. Similar results were found in in $CD4^+CD25^+$ T cells from B6 mice that expressed that same class II allele ($B6^{g7}$ mice). We had previously shown that $CD4^+CD25^+$ T cells from pre-diabetic NOD mice also expressed an intermediate expression of Foxp3 (Manirarora 2008). Similarly, the ability of APC from diabetes-resistant congenic and pre-diabetic NOD mice to maintain Foxp3 expression also was intermediate suggesting that the MHC class II I-$A\beta^{g7}$ allele is involved at some level in the decrease in Foxp3 expression in $CD4^+CD25^+$ T cells from NOD mice. The MHC class II I-$A\beta^{g7}$ allele is one of multiple genetic loci that control disease susceptibility in NOD mice. It has been reported that coexpression of protective βchains such as I-$A\beta^d$ or I-$A\beta^k$ with the endogenous disease-prone βchain (I-$A\beta^{g7}$) in about 15% of bone marrow-derived haematopoietic stem cells provides protection against the development of insulitis and diabetes (Tian 2004). However, the MHC class II I-$A\beta^{g7}$ allele is not sufficient to induce disease as mice expressing the defective MHC-Class II allele I-$A\beta^{g7}$ on a C57BL/6 (B6) background ($B6^{g7}$ mice) do not develop diabetes (Wong 2005). Furthermore, the ability of APC to maintain Foxp3 expression is lower if $CD4^+CD25^+$ regulatory cells from sick NOD mice are used in the *in vitro* assay compared with pre-diabetic NOD mice, suggesting that the disease state can affect Foxp3 expression in $CD4^+CD25^+$ regulatory cells at some level. Using transgenic mice that were rendered chronically hyperglycemic beginning shortly after birth by rat insulin promoter driven expression of calmodulin (OVE26) and subsequent β cell damage (Epstein 1989), we showed that higher glucose levels have no effect on Foxp3 expression in $CD4^+CD25^+$ regulatory cells (Manirarora 2008). However, other metabolites that are produced during diabetes progression may affect APC and/or regulatory cells, thereby leading to further decreases in Foxp3 expression.

We had shown previously that activation of APC *in vivo* by treatment with CFA appeared to significantly enhance the NOD APC's ability to activate $CD4^+CD25^+$ regulatory T

cells, as indicated by increased regulatory activity both *in vivo* and *in vitro*, and restoration of an optimal regulatory cell phenotype, i.e., increased Foxp3 expression in $CD4^+CD25^+$ T cells (Manirarora 2008). Our data suggested that the effect of CFA was, at least in part, mediated through enhancement of the ability of NOD APC to act on NOD $CD4^+CD25^+$ regulatory T cells by sustaining Foxp3 expression. In the current manuscript we demonstrated that APC stimulated by CFA *in vivo* could indeed induce induction of Foxp3 expression in NOD regulatory cells *in vivo*, by performing a local adoptive transfer of CFA-treated APC and assessing Foxp3 expression in the draining LN a few days later. Because CFA, which contains desiccated *Mycobacterium tuberculosis*, appears to mediate its effect *in vivo* through TLR2 (Lim 2002), we tested whether a TLR2 ligand could reproduce the CFA effect *in vitro*. Pre-treatment with lipoteichoic acid (LTA), a TLR2 ligand, could indeed restore the ability of NOD APC to maintain Foxp3 expression in $CD4^+CD25^+$ regulatory cells *in vitro*, indicating that cell wall components targeting TLR are capable of mediating this effect. Another cell wall component of *Mycobacterium tuberculosis*, mannose-capped lipoarabinomannan, has been shown to induce expansion of $CD4^+CD25^+Foxp3^+$ regulatory T cells by binding to receptors expressed by APC (Garg 2008). There are, therefore, several potential candidates capable of enhancing the ability of APC to preferentially induce regulatory cells.

In conclusion, we have established a link between the decreased expression of Foxp3 in $CD4^+CD25^+$ regulatory cells and the expression of MHC class II I-$A\beta^{g7}$ allele by APC in NOD mice. Moreover, disease progression appear to affect Foxp3 expression as well. More importantly, the ability of NOD APC to induce/maintain Foxp3 expression in $CD4^+CD25^+$ T cells can be restored by stimulation with TLR ligand, and manipulating the ability of APC to activate/induce $CD4^+CD25^+$ regulatory T cells is a potential strategy that could be used to prevent disease.

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Advantage of Organic Supplementation of Inorganic Fertilizer in Walleye (*Sander vitreus*) Hatchery Ponds at Minor Clark Fish Hatchery, KY

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ABSTRACT

We used inorganic fertilizer in three ponds at the Minor Clark Fish Hatchery and compared results with three ponds treated with a traditional combination of organic and inorganic fertilizer. Organic augmentation resulted in significantly higher water concentrations of chlorophyll *a* (16 vs. 7 μg L^{-1}), total P (167 vs. 89 μg L^{-1}), SRP (9.3 vs. 6.6 μg L^{-1}), and inorganic nitrogen (230 vs. 184 μg L^{-1}). Secchi depth and DO (7.9 vs. 11.2 mg L^{-1}) were lower in the organically fertilized ponds. No filamentous algal blooms occurred in any ponds. Rotifers were the predominant zooplankton in all ponds; but traditionally fertilized ponds had greater copepod biomass. Fish survival (37%) and yield (36 kg ha^{-1}) in inorganically fertilized ponds were much lower than previously found at Minor Clark Hatchery (about 90% survival). Although use of inorganic fertilization alone may reduce fertilizer and supplemental aeration costs in some hatcheries, it did not offset lost fish production in this study.
KEY WORDS: Fish production, plankton, productivity, fertilizer, algae

INTRODUCTION

Inorganic fertilizers have been used to significantly increase walleye [*Sander vitreus* (Mitchill)] survival and growth when added to hatchery ponds as a nutrient source (Qin and Culver 1992) or to augment organic fertilizer such as chopped hay or plant meals (Fox et al. 1989; Myers et al. 1996). Inorganic fertilizers commercially available liquid or solid N-P-K) allow more precise application rates than organic fertilizers but do not provide an organic carbon supplement that may increase microbial and protozoan growth. Organic fertilizers provide organic supplementation to feed microbes and some zooplankton and release algal nutrients more slowly; however, decomposition may deplete the pond of dissolved oxygen.

A number of previous studies have examined optimizing pond fertilization using inorganic fertilizers alone, or as a supplement to organic fertilizers. Culver (1991), based upon the resource ratio hypothesis (Smith 1983), found an N:P ratio of 20:1 increased growth of palatable pond algae and reduced growth of nuisance species resulting in increased survival and production of walleyes. Soderberg et al. (1997) concluded that inorganic fertilizer should be used instead of organic fertilizers because it is cheaper but equal to or greater in efficiency. Middleton and Reeder (2003) found that by eliminating organic fertilizers in walleye ponds, lethal dissolved oxygen (DO) declines were less common.

Inorganic fertilizer has been found to reduce survival but not necessarily production, when walleye fry are stocked at 50 fish m^{-3} (Myers et al. 1996; Tice et al. 1996). There is some suggestion that desirable zooplankton produced by inorganic fertilization may be reduced by top-down fish predation (Barkoh 1996; Qin and Culver 1996). Barkoh (1996) found that fish survival was lower in striped bass ponds receiving only inorganic fertilizer; Boyd (1990) and Kurten et al. (1999) suggest that carbon supplied by organic fertilizer was necessary to sustain optimal pH and algal productivity in aquaculture ponds.

Inorganic fertilization alone may provide the benefits of organic fertilization without drawbacks; however as noted above, performance has been mixed. Inorganic fertilization is usually used in an attempt to increase fish

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production in hatcheries with a history of poor yields. The purpose of this study was to compare the effects of inorganic fertilization with traditional organic plus inorganic fertilization on water quality, plankton, and fish production in hatchery ponds with a history of high survival and production.

MATERIALS AND METHODS

Minor E. Clark Fish Hatchery (MCFH) in Morehead, Kentucky, is located in the tailwater area of Cave Run Lake and covers 122 ha of the Licking River alluvial flood plain. The 0.41 ha surface area ponds have sloped bottoms with a maximum depth of 1.5 m and a minimum depth of 0.6 m. Oligotrophic Cave Run Lake water (Davis and Reeder 2001) can be drawn from different depths to control temperature and dissolved oxygen (DO) when filling ponds. Three randomly assigned ponds were fertilized with an organic and inorganic mix employed at the hatchery (Traditional), and three were treated only with inorganic fertilizer (Experimental). Ponds were stocked with 110,000 (about 268,000 ha^{-1} or 29 fish m^{-3}) 5-day old fry spawned from local brood stock from east Kentucky reservoirs on 1 May 1997.

Fish counts were determined by water displacement. Walleye populations in each pond were seined three times during the experiment to determine growth rates and to establish harvest times. Walleye collected were measured (length and weight) and health conditions evaluated.

Historically, ponds at Minor Clark Fish Hatchery have been fertilized with both organic and inorganic fertilizer. During our study, Traditional ponds received an initial fertilization of 4 bales of chopped hay, 181 kg of soybean meal, 68 kg of alfalfa meal, 2.7 kg of potash, and 454 kg of lime prior to stocking. Over the course of the study, 642 kg (107 kg) of alfalfa meal were added to each pond per week and 3.79 L of 9-18-9 liquid fertilizer for three weeks after stocking, as well as 1.36 kg of potash three weeks after stocking.

Experimental ponds were not augmented with organic fertilizers and were treated only with inorganic nutrients. Three Experimental ponds were filled with water immediately before stocking. Weekly fertilization rates were determined based upon N and P concentrations measured prior to application. Inorganic nitrogen (NH_4 and NO_3) concentrations were raised to 0.6 mg L^{-1} and soluble reactive phosphorous (SRP) concentration to 0.03 mg L^{-1}. Weekly fertilization rates averaged 4.61 L of 28-0-0 and 0.13 L of phosphoric acid.

Fertilization and sampling occurred from April through May. Each pond was sampled biweekly at two sites and three depths (surface, middle, and bottom) with a 2-L Van Dorn bottle. Secchi depth was determined with a 20-cm black and white Secchi disk. Duplicate analyses were run on all water samples. Water quality data were reported as the mean of combined samples per pond. Normally, fish are held in ponds for five to six weeks to allow them to reach harvestable size. Due to cooler than average water temperatures, fish required about 8 weeks to reach a harvestable size.

Chlorophyll *a* was extracted using 90% alkaline acetone from filtered water (0.45 µm Whatman GF/A glass fiber filters) and analyzed flourometrically (USEPA 1979). Alkalinity was measured by titrating with 0.02 *N* H_2SO_4 using an ASTM certified class A burette (precision ± 0.02 ml) to a pH = 4.8 (Larson and Henley 1955). Dissolved nutrients were determined from filtered water. NO_3 was analyzed using the sulfanilamide method following cadmium reduction (Henrickson and Selmer-Olsen 1970); ammonium (NH_4) was determined using a Nesslerization technique (Jenkins 1967); soluble reactive phosphorus (SRP) was measured using the ascorbic acid method (Murphy and Riley 1962). Total phosphorus was determined as SRP from unfiltered water following a persulfate digestion (USEPA 1979). All water samples were analyzed within 24 h of collection.

The plankton community was sampled at each site with a 12-L Schindler-Patalis Plankton trap with a 20 µm mesh. Plankton were washed into a 125 ml Nalgene bottles, preserved with 4% sweetened formalin and Lugol's iodine, and kept refrigerated at 4°C until they were enumerated and identified. Ecosystem metabolism was assessed using Dawn-Dusk-Dawn dissolved oxygen (DO) changes; DO readings were recorded at each site and depth with an air-calibrated YSI® Model 54 DO meter.

Differences in water quality and productivity averages were assessed using a *Student's t*-test after determining the data were normally distributed. The variability among replicate ponds on any given day was too great to allow

Table 1. Mean water quality, survival, and yield in walleye hatchery ponds at Minor Clark Fish Hatchery

Treatment	Secchi depth (m)	Chlorophyll a $\mu g\ L^{-1}$	DO $mg\ L^{-1}$	Alkalinity $mg\ CaCO3\ L^{-1}$	Inorganic N $\mu g\ L^{-1}$	SRP $\mu g\ L^{-1}$	TP $\mu g\ L^{-1}$	Survival %	Yield $kg\ ha^{-1}$
Traditional	0.72	15.89	7.91	57.61	230.1	9.3	167.2	88	79
Experimental	1.15	6.64	11.21	34.05	183.5	6.6	89.1	37	36

for comparisons between treatments on a day-to-day basis; therefore, data were averaged over the course of the experiment. Measurements that should be related, but independent, were compared with correlations. Correlation significance was assessed with a paired z-test. We chose a 0.05 level of significance for all statistical analysis. Means are reported ± standard error. Statview 4.0 for Macintosh (Abacus Concepts, Inc. 1992) was used to calculate all statistics. We compared only paired data from days when all ponds had water and were actively growing fish and being fertilized.

RESULTS

Secchi transparency was significantly higher ($P < 0.05$, N = 72) in the Experimental ponds compared with the Traditional ponds (Table 1, Figure 1). Source water (Cave Run Lake) alkalinity is usually around 25 mg $CaCO_3\ L^{-1}$ (Davis and Reeder 2002). Organically fertilized ponds had nearly double the amount of dissolved carbon ($P < 0.05$, N = 72). Control pond mean averages were 57.6 mg $CaCO_3\ L^{-1}$; Experimental ponds were slightly higher than source water (34.1 mg $CaCO_3\ L^{-1}$). Alkalinity gradually increased over the course of the experiment in both pond types.

Daily maximum pH and minimum pH values (measured at dusk and dawn respectively) were significantly different ($P < 0.05$, N = 30) for the Experimental verses the Traditional ponds. Experimental pond low and high pH means were 9.10 and 9.48, respectively, while the Traditional pond means were 7.89 and 8.55. Mean dissolved oxygen (DO) concentrations were significantly higher ($P < 0.05$, N = 684) in the Experimental ponds than in the Traditional ponds.

Experimental ponds never had less than 4 mg L^{-1} DO; Traditional pond bottom waters were less than 4 mg L^{-1} in 22% of the bottom samples. Experimental ponds mean DO was 10.57 mg L^{-1} at the surface and 9.97 mg L^{-1} at the bottom at daybreak and rose to about 12 mg L^{-1} throughout the water column near sundown. Traditional ponds average 7.55 mg L^{-1} DO at the surface and 5.15 mg L^{-1} near dawn, and whole-ecosystem photosynthesis exceeded respiration to bring dusk concentrations to 10.38 mg L^{-1} at the surface and 6.78 near the sediment. One Traditional pond within this study required supplemental aeration.

Dissolved oxygen values less than 4 mg L^{-1} were not as common as in previous years in any of the hatchery ponds due to cooler than normal air temperatures. Pond temperatures ranged from 9°C (when the ponds were filled with stream water) to 20°C over the course of the experiment. Temperatures rose to 13°C within one week after filling. April mean air temperatures were 2.7°C below normal, while May temperatures were 4.0°C below the 30-y average.

Nutrient concentrations in ponds augmented with organic fertilizer were always higher than in ponds with only inorganic fertilization; however, none of the ponds had extraordinarily high levels of nutrients found in some hatcheries because the source water is relatively clean. Nitrate and NH_4 were never high enough to create toxicity problems, and NO_3 was more abundant than NH_4. Total inorganic nitrogen concentrations (NO_3 + NH_4) were not significantly different between the two pond types. Nitrite means were not significantly different between the two treatments.

The ratio of total inorganic nitrogen to SRP was always above 20:1. Concentrations of total phosphorus were more than an order of magnitude greater than SRP. Experimental pond total phosphorus concentrations were significantly higher post-fertilization compared with pre-fertilization ($P < 0.05$, N = 72). Soluble reactive phosphorus was low in all ponds and not significantly different between the two pond types.

Chlorophyll *a* concentrations were not extraordinary in any pond, but they were significantly lower in the Experimental ponds ($P < 0.05$, N = 72; Table 1). Algal growth generally increased in both treatments

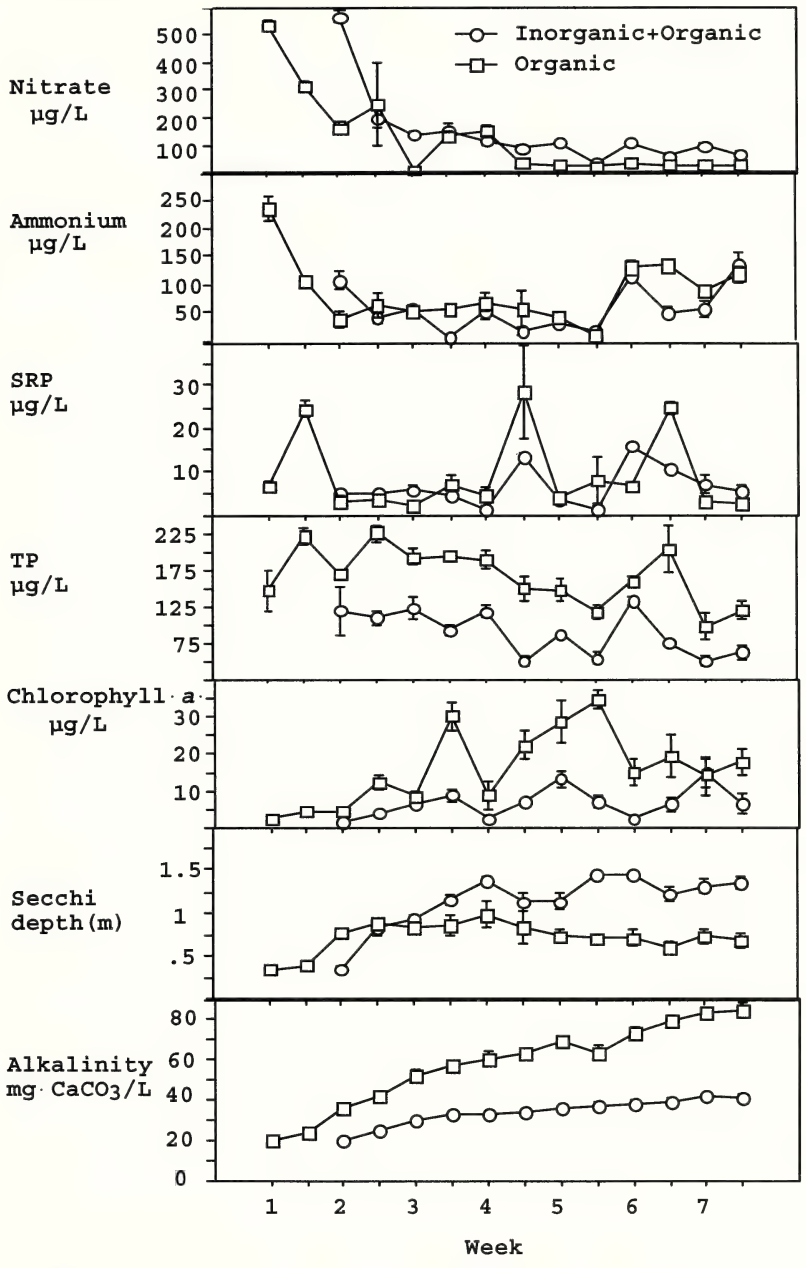

Figure 1. Water quality changes over the 1997 growing season in Minor Clark Fish Hatchery ponds. Bars represent one standard error.

throughout the course of the experiment (Figure 1). Higher primary production in the Traditional ponds resulted in lower water transparency.

There were few differences in the phytoplankton communities of the ponds: Green algae and diatoms dominated the phytoplankton throughout the experiment (Figure 2). Prior to walleye stocking, Green algae dominated, with most of the remainder comprised of diatoms. The initial phytoplankton community was similar to that found by Davis (1995) in Cave Run Lake. Following stocking, diatoms became the dominant group for weeks three and four; green algae (81% to 92%) dominated weeks six through eight. No

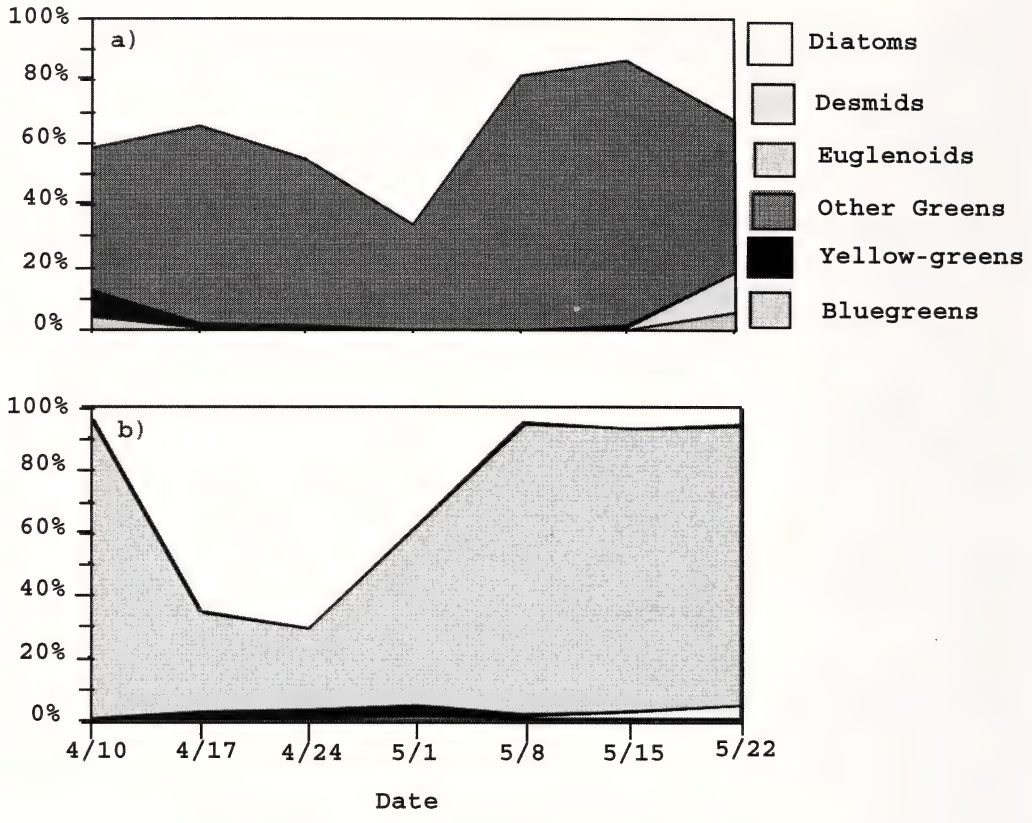

Figure 2. Phytoplankton relative abundance in (a) Traditional organic + inorganically fertilized ponds and (b) Experimental inorganically fertilized ponds at Minor Clark Fish Hatchery.

pond in this study experienced nuisance growth of undesirable colonial blue-green algae.

The ponds had markedly different zooplankton communities (Figure 3). Zooplankton populations within the Traditional ponds prior to stocking were comprised of copepod napulii (39%), rotifers (34%), copepods (18%), cladocerans (8%), and ostracods (1%). The zooplankton community in organically fertilized ponds resembled that of the source water (Davis 1995), with rotifers dominant from week two until harvest, ranging from 54% to 98% of the community. Nauplii comprised 4% to 13% week two through week seven. The only exception to rotifer domination occurred during weeks four through seven when a large bloom of copepods appeared as the nauplii matured.

Total mean numbers of zooplankton peaked twice within the Traditional ponds, on May 1 at 12,282 individuals L^{-1} and May 22 at 7654 individuals L^{-1} (Figure 4). Both peaks were dominated by rotifers (10,810 individuals L^{-1} and 7485 individuals L^{-1} respectively). The total mean numbers of zooplankton within the Experimental ponds gradually increased until they peaked on the last sample date (May 22) at 9135 individuals L^{-1}. The peak was dominated by rotifers (8,534 individuals L^{-1}). Zooplankton density was greater within the Traditional ponds until May 8, when their numbers dropped to 895 individuals L^{-1}, compared with 3,331 individuals L^{-1} within the Experimental ponds. The mean zooplankton density was higher within Experimental ponds until walleye harvest.

Rotifers dominated the community structure within the Experimental ponds. Initially, rotifers comprised 55% of the zooplankton population, with cladocerns and larval napulii making up another 39%. A week later, the population was 87% rotifers with the majority of the remainder comprised of napulii. This contrasted with source water in early May where Davis and Reeder (1995) found Cave

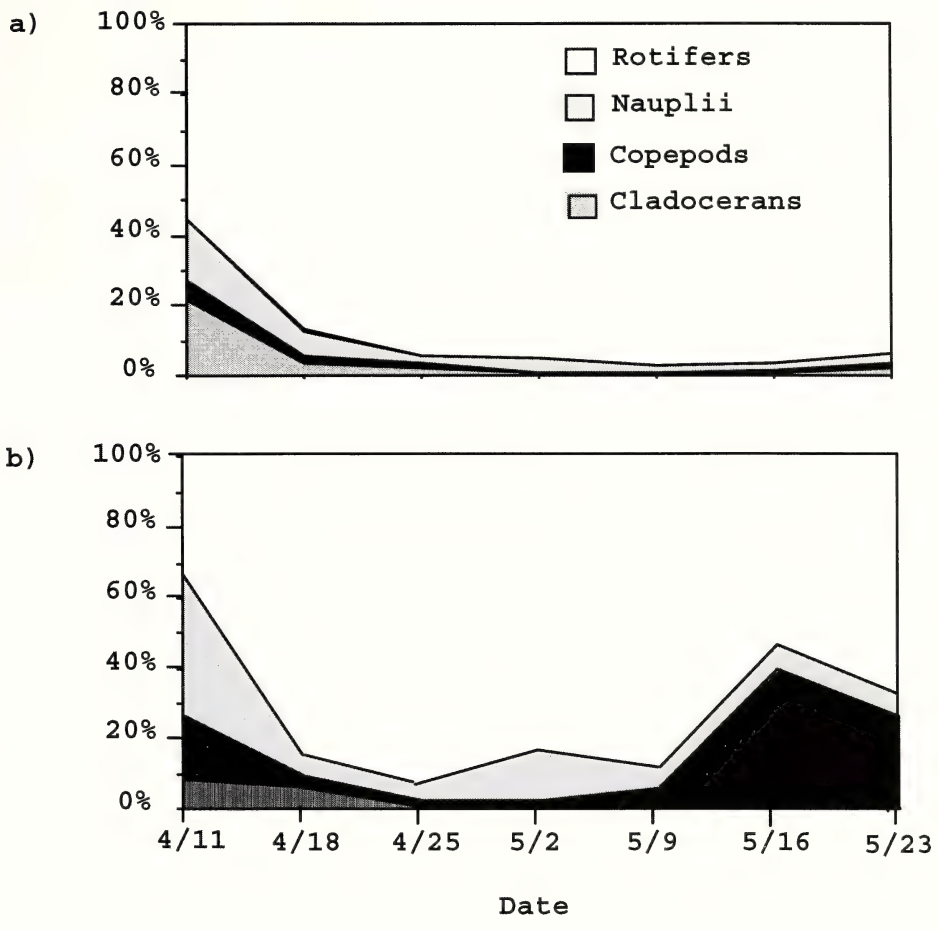

Figure 3. Zooplankton relative abundance in (a) Traditional organic + inorganically fertilized ponds and (b) Experimental inorganically fertilized ponds at Minor Clark Fish Hatchery.

Run Lake populations comprised of adult copepods (24%), napulii (14%), cladocerns (30%), rotifers (30%), and ostradcods (2%).

Walleye survival and production was higher in Traditional ponds ($P < 0.05$, N = 3). Walleye survival averaged 37.3% in Experimental ponds and 86.7% in Traditional ponds. Accordingly, production was 36.32 kg ha^{-1} in the Experimental ponds compared with 78.76 kg ha^{-1} in Traditional ponds. Average walleye harvested from Traditional ponds were shorter (36.41 mm) and heavier (611.0 fish kg^{-1}) than those from Experimental ponds (38.61 mm and 552.0 fish kg^{-1}).

DISCUSSION

At Minor Clark Fish hatchery, the elimination of organic fertilizer provided many of the benefits reported at other hatcheries: elimination of problem anoxia; lack of noxious algal blooms, and reduced cost. Traditional ponds did not experience problems with cyanobacteria blooms, and only one was close to anoxic for a short time; however, other organically fertilized ponds at Minor Clark Fish Hatchery required both supplemental aeration and $CuSO_4$ treatments.

Minor Clark Fish Hatchery has been using a combination of organic and inorganic fertilizer to rear walleye for over a decade, and fish survival rates average 89%, which is higher than most hatcheries. Inorganic fertilizer alone was incapable of supporting and maintaining ecosystem energy required for fish survival. The clear waters of the Experimental ponds had more DO but did not have sufficient plankton densities to support the

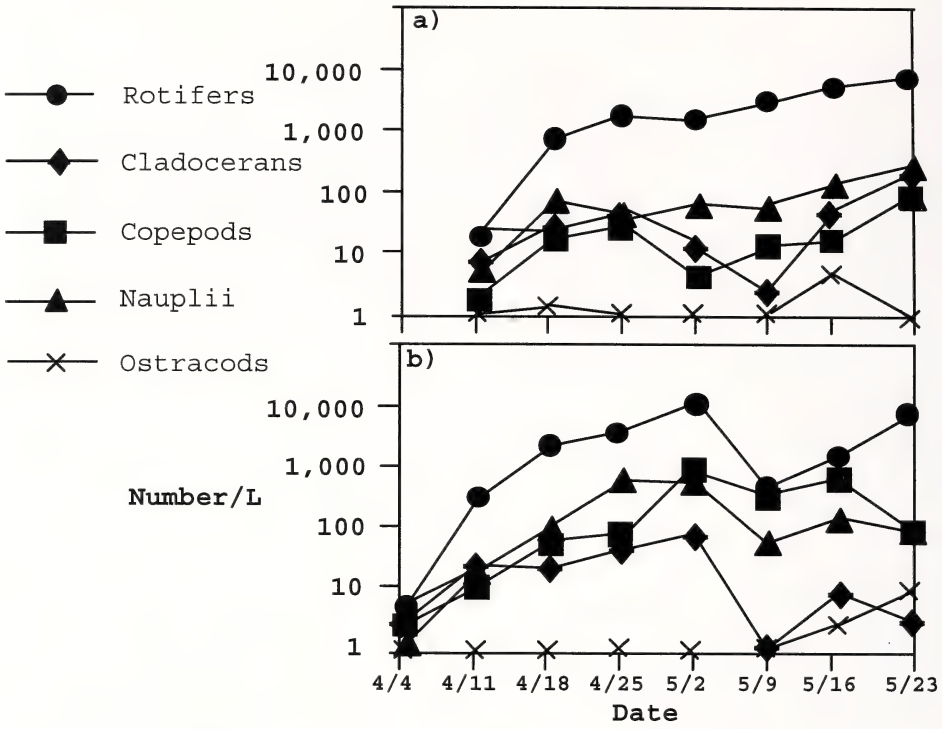

Figure 4. Zooplankton abundance in (a) Traditional organic + inorganically fertilized ponds and (b) Experimental inorganically fertilized ponds at Minor Clark Fish Hatchery.

stocked walleye fry populations. Results from this limited trial did not support the idea that there are similar yields without supplementing inorganic fertilizer with organic fertilizer.

During the study year, holding times were two to three weeks longer than the month normally required for walleye to reach the desired size for waterway stocking. Given this longer holding time, we would expect to see an increase in fish mortality compared with other studies. Algal production and TP concentrations were similar or higher to those found in other studies (Schroeder 1978; Qin and Culver 1992, 1995; Myers et al. 1996; Tice et al. 1996; Tew et al. 2006). Total phosphorous concentrations within Experimental ponds were lower than those achieved by Qin and Culver (1995); and TP in Traditional ponds was higher than in Experimental ponds. Although TP concentrations were nearly double in Traditional ponds, Experimental ponds averaged more than 89 μg L^{-1} TP; therefore, there was always more total phosphorus in both pond treatments than required to support the algal biomass measured (using models of Vollenweider or Carlson). We also met our goal of keeping the N:P ratio high, as recommended by Culver et al. (1993).

There is no conclusive evidence that lack of nutrients (bioavailable P, N, or C) caused the reduced fish production. Qin and Culver (1992) found mean SRP concentrations of 12.40 μg L^{-1} and 14.86 μg L^{-1} within ponds fertilized with inorganic fertilizers only, and inorganic and organic fertilizers combined, while this research obtained 6.6 μg L^{-1} and 18.0 μg L^{-1} respectively. At Hebron Fish Hatchery in Ohio, Tew et al. (2006) found that bringing pond SRP concentrations up to 20 μg L^{-1}, rather than 30 μg L^{-1}, did not result in reduced fish survival or production but did reduce algal biomass. Similarly, Soderberg et al. (2000) found that increasing nutrients (N, P, and inorganic C) did not increase juvenile walleye production.

Lowering NH_4 concentrations is beneficial in hatchery ponds because it diminishes the possibility of fish-toxic concentrations of undissociated ammonia forming under the high pH created by algal metabolism.

Because there was less organic matter decomposition and system respiration (Middleton and Reeder 2003), ammonia was significantly ($P < 0.002$, N = 72) lower in the Experimental ponds. Qin and Culver (1992) also found NH_4 concentrations were significantly reduced in ponds fertilized with only inorganic fertilizers as compared with those fertilized with inorganic and organic fertilizers. The NH_3 levels should confirm this for toxicity; however, lower ammonia concentrations did not lead to greater fish survival in our Experimental ponds.

There is circumstantial evidence that the Experimental ponds had insufficient carbon. Mean alkalinity in Experimental ponds was 34 mg $CaCO_3$ L^{-1} and average nearly 58 mg $CaCO_3$ L^{-1} in Traditional ponds. Although the correlation between alkalinity and chlorophyll *a* was not significant ($P > 0.05$, N = 24), overall, ponds with the highest alkalinity had the highest productivity in both plankton and fish. The pond with the lowest alkalinity had the lowest productivity and was a "bust." Boyd (1997) suggested lakes with alkalinity less than 20 mg $CaCO_3$ L^{-1} will not have sufficient carbon for production, and highly productive ponds may require up to 100 mg $CaCO_3$ L^{-1}. In intensive aquaculture systems it is common to add lime or organic fertilizer to increase carbon available for primary production (Biro 1995). Tice et al. (1996) suspected low alkalinity limited walleye production in inorganically fertilized ponds at Pleasant Mount Fish Cultural Station in Pennsylvania; however, the carbon dynamics in hatchery ponds probably are complex.

If carbon were limiting at Minor Clark, we would have expected to find a greater correlation between carbon and chlorophyll or net primary production in the ponds with low alkalinity compared with high alkalinity. Chlorophyll concentrations in the organically fertilized ponds were lower at high alkalinity. Soderberg et al. (2000) did not find liming enhanced production of walleye in low alkalinity water ponds. Other carbon interactions, such providing substrate for the growth of bacterial and protozoan communities also could have contributed to increased fish and plankton production in the organically fertilized ponds.

Culver (1991), based upon the resource ratio hypothesis (Smith 1983; Tilman et al. 1986) hypothesized that filamentous algae would be reduced by maintaining a high N:P (>20:1) ratio. Maintaining the high N:P has worked well in Culver's pond studies (Tew et al. 2006). Similarly, we found phytoplankton communities were dominated by green algae and diatoms within both pond types throughout the course of this experiment. Unpalatable algae dominance was not a problem within either pond type at any time. However, Tice et al. (1996) experienced fish loss due to entanglement within filamentous algae in ponds utilizing Culver et al. (1993) methods.

Total numbers of phytoplankton cells within the Experimental ponds were about 27% lower than within the Traditional ponds. This plankton shortage could have been one of the problems with utilizing this fertilization program at MCFH this year. Because large zooplankton were not present in any pond, it was unlikely that grazing caused reduction of algal cells. It could be that turbidity or normal seasonal variations resulted in significantly less algal biomass in Experimental ponds.

Rotifers are the preferred food of fry when they are less than a week old, but fry should quickly move up to larger zooplankton as they grow. Both fertilization types resulted in similar zooplankton blooms (dominated by rotifers early within the experiment). Walleye forage optimally (Graham and Sprules 1992; Qin and Culver 1992), thus they probably do not select for rotifers if larger prey are available. We assume fry were selectively feeding on larger plankton and leaving smaller prey such as rotifers. Traditional ponds experienced a copepod bloom later into the study, which indicated that there was sufficient desirable food available in these ponds. Experimental ponds quickly became dominated by rotifers (one week after stocking, 87%), suggesting possible food limitations in these ponds.

A possible critical food web dynamic not measured in this study is the contribution of the protozoan community to the energy flow in these pond ecosystems. Organic fertilizer could increase microbial and protozoan production. A protozoan community, enhanced by organic fertilization, could be providing an extra energy pathway (apart from algae) that resulted in increased fish production and survival.

The cool temperatures nearly doubled the time it took fish to grow to harvestable size.

This extended holding time may have increased chances for fish mortality. Soderberg et al. (2000) suggest early season temperature variations, such as our ponds experienced, are the cause of mortality and reduced production in walleye ponds—not lack of nutrients.

The primary goal of hatchery managers is to achieve optimal fish production within a given pond without undue stress upon the fish at the lowest cost. Both types of treatments (Experimental and Traditional) seemed to provide enough important nutrients to ensure that sufficient plankton blooms developed to promote fish growth. Less time and money was required to fertilize the Experimental ponds. Using only inorganic fertilization could result in annual savings; however, other costs associated with the Experimental fertilization program (water quality monitoring, reduced fish production) did not make it cost effective in the present study.

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NOTE

Induction of Calcium Uptake in Erythrocytes by Agricultural Chemicals—Calcium has important functions in many biological systems and exerts control over a wide range of cellular processes (Jaiswal 2001). Any impairment of mechanisms that regulate intracellular calcium within a narrow physiological range can increase cytosolic calcium concentrations (Hurwitz 1996). Cell calcium uptake can be initiated by the inhibition of calcium adenosine triphosphatases (ATPases) in the plasma membrane, the reticuloendothelial system, and mitochondria (Carafoli 1987). In addition, oxidative stress, hypoxia, or xenobiotics are known to elevate calcium concentrations in the cytoplasm, which in turn modify cellular responses and eventually lead to cytotoxicity and cellular dysfunction (Nicotera et al. 1992; Orrenius and McCabe et al. 1992). Human erythrocytes are a relevant model system to study plasma membrane ion transport into cells because they lack organelles. FURA 2 AM is a cell permeant fluorescent calcium binding dye that is used to determine intracellular calcium concentrations (Villereal and Palfrey 1989). This note reports on the ability of some agricultural chemicals to initiate calcium uptake by human erythrocytes, in vitro, using FURA 2AM with the aim of identifying an indicator of pesticide exposure in occupationally exposed farm workers.

Erythrocytes were collected from freshly drawn heparinzed blood donated by urban volunteers not known to have been exposed to pesticides. The erythrocytes were suspended in 10 mM HEPES buffer, pH 7.4, containing 80 mM KCl, 70 mM NaCl, and 0.15 mM $MgCl_2$ (HEPES). Glucose (10 mM), penicillin (100 units/100 mL) and streptomycin (1 mg/100mL) were added to provide energy and preserve the cells. The cells were stored for no more than 3 days in the refrigerator and used for calcium uptake studies by the method of Grynkewicz et al. (1985). FURA 2AM (Molecular Probes, Eugene, OR) was dissolved in dimethyl sulfoxide to obtain a stock concentration of 50 mM, and 200 µL aliquots were stored at −80°C. The FURA 2AM stock was diluted just before an experiment to 2.5 µM with HEPES buffer containing 10 mM glucose and 5% bovine albumin (buffer B). A known volume of erythrocytes was suspended in 20 mL buffer B and incubated in a shaker bath at 37°C for 30 min after the addition of FURA 2AM indicator. These cells were washed twice with HEPES buffer after the incubation medium was discarded and re-incubated at 37°C with buffer B for 30 min to hydrolyze FURA 2AM absorbed by the cells to FURA 2. Ten replicates of aliquots of FURA 2 loaded erythrocytes in 10 mL buffer B (10^6 cells) were incubated at 37°C for 30 min with and without 10 mM Ca^{2+} ($CaCl_2$ solution) and varying levels of the chemicals made up in HEPES buffer: acephate (*OS*-dimethyl acetyl phosophoramidthioate); 1.0, 2.5, 5 M; maleic hydrazide (1,2-dihydro-3,6-pyridazinedione):0.05, 0.1, 0.25 M; nicotine: (S-3-(1-methyl-2-pyrrolidinyl)-pyridine): 0.25, 0.5 and 1.25 M. Nicotine was included in this study as it has been used as a pesticide, and farm workers are exposed to it in the tobacco growing regions of the US. At the end of the 30 min incubation period the cells were centrifuged and washed with HEPES after the incubation medium was discarded, resuspended in HEPES buffer, and scanned in a spectrofluorometer (SPEX, model FluoroMax 2, Edison, NJ). Of the chemicals, only maleic hydrazide had fluorescence of its own at a wavelength range (>380 nm) outside that of FURA 2 and thus did not interfere with the calcium uptake measurements. The treated cells with corresponding blanks (blanks in the absence or presence of each chemical and calcium) and controls were scanned at excitation wavelength between 300 to 400 nm and emission kept at 510 nm. The ratios of peaks at 336 (with Ca^{2+}) and 364 (control without 10 mM Ca^{2+}) were used to calculate intracellular Ca^{2+} uptake by erythrocytes (Table 1).

Figure 1 shows an example of a FURA 2 scan; scans of cells incubated with nicotine and with and without Ca^{2+} are superimposed in this Figure for comparison. In this example, the effect of 0.25 M nicotine on peak shift to 336 nm from 364 nm after addition of Ca^{2+} is apparent and indicates calcium uptake by the cells. The agricultural chemicals induced varied uptake of Ca^{2+}. The average uptakes were (n = 10) acephate 1.0 M = 176 ± 65 nM; maleic hydrazide 0.05 M = 44 ± 16 nM and nicotine 0.25 M = 78 ± 24 nM. The addition of increasing levels of the three chemicals did not stimulate further increase or uncontrolled uptake of Ca^{2+} by the erythrocytes (Figure 2).

Some organochlorines such as chlorophenols, lindane, and endosulfan are reported to inhibit Ca^{2+} transport ATPases that are calmodulin dependent in human erythrocyte membrane preparations at low concentrations that elevate intracellular calcium (Janik and Wolf 1992). In addition, some chlorinated pesticides appear to increase calcium in rat myometrial smooth muscle cells by inducing the influx of calcium through voltage-dependent calcium channels (Juberg et al. 1995). Casares and Mantione (2006) have suggested that alteration of intracellular Ca^{2+} transients by pesticides and other environmental pollutants may represent an important common mechanism responsible for the negation of constitutive nitric oxide synthase activation that could compromise health by altering immune, vascular, and neurological functions. Another mechanism is the inhibition of endogenous antioxidant superoxide dismutase (SOD) that results in the accumulation of oxygen free radicals in cells that in turn induces calcium uptake (Nicotera et al. 1992). This may be particularly so in erythrocytes that have the major function of transporting oxygen to the whole body and thus have high levels of several antioxidant enzymes such as catalase and glutathione peroxidase in addition to SOD and have high

Table 1. Effect of pesticides on the induction of calcium uptake by human erythrocytes, in vitro.

Excitation wavelength	Pesticide		Blank counts*	Ratio	− Calcium counts*	Ratio	+ Calcium counts*	Ratio	R-Rmin Rmax-R
nm	M		R		Rmin		Rmax		Ca, nM
336	Acephate, 1		44013		38421		40486		
				0.62		0.59		0.65	135
364			70402		64932		62128		
336	Maleic	0.05	12053		2344		8629		
	Hydrazide			0.06		0.02		0.14	72
364			2.079×10^5		80697		63794		
336	Nicotine	0.25	1.63×10^5		5.74×10^5		5.63×10^5		
				0.77		0.70		0.96	71
364			2.11×10^5		7.70×10^5		5.88×10^5		

* Fluorescence counts; emission wavelength was kept at 510 nm. R = Blank ratio - no pesticide or Ca; Rmin = ratio with pesticide; Rmax = ratio with Ca and pesticide.

concentrations of the redox molecule glutathione to maintain redox status. We found that the chemicals tested in this study also inhibited human erythrocyte SOD activity both in vitro and in vivo (Dowla et al. 1996; Panemangalore et al. 1999; Tope and Panemangalore 2006). We attempted to determine the effect of verapamil, a Ca channel blocker, on Ca uptake in our experiments but hemolysis of the erythrocytes prevented further investigation (Watts and Handy 2007). Although, the chemicals we tested could disrupt calcium homeostasis in erythrocytes by one of the three mechanisms, it is possible that inhibition of SOD was responsible for the transient influx of calcium into human erythrocytes in the present study. The absence of increased calcium influx at higher levels of the chemicals supports this observation. The results also suggested that calcium concentrations of erythrocytes cannot be used as an indicator of exposure to agricultural chemicals.

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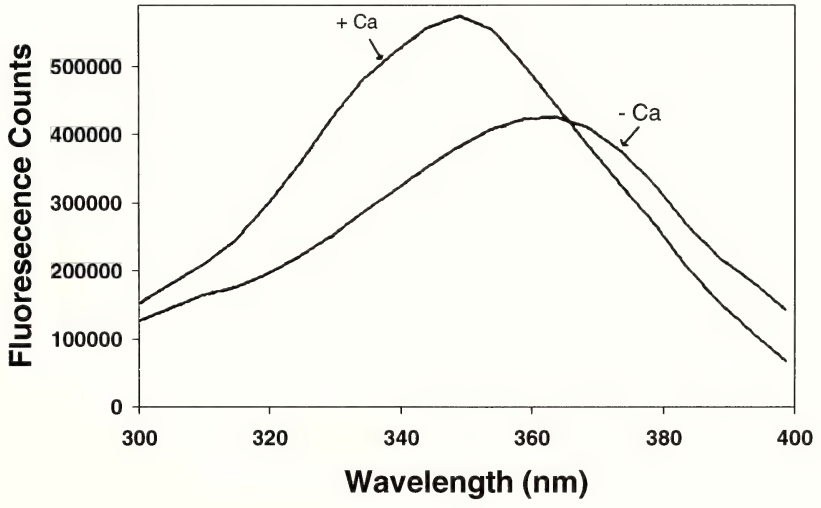

Figure 1. Fluorescent scans of FURA 2 loaded erythrocytes after exposure to nicotine (0.25 M) with and without 10 mM Ca^{2+} for 30 min. Intracellular saturation with calcium shifted the scan to peak at about 336 nm as compared with a peak at of 364 nm without calcium.

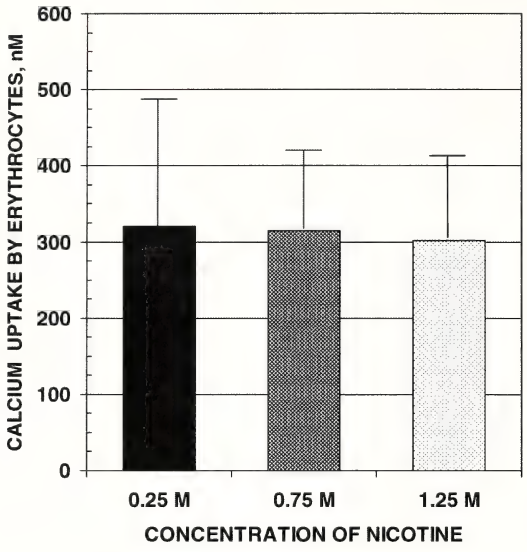

Figure 2. The effect of increasing concentrations of nicotine on influx of calcium into erythrocytes after 30 min exposure. Mean ± SD; n = 10.

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Abstracts of Some Papers Presented at the 2009 Annual Meeting of the Kentucky Academy of Science

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AGRICULTURAL SCIENCES

Bensulide Residues in Runoff Water from Agricultural Soil. GEORGE F. ANTONIOUS, Kentucky State University, Land Grant Program, Frankfort, KY 40601.

Bensulide [O, O-diisopropyl S-2-phenylsulfonylaminoethyl phosphorodithioate] is one of the few herbicides from the organophosphate group used for control of weeds that threaten numerous crops. A field study was conducted on a silty-loam soil of 10% slope at Kentucky State University Research Farm to monitor off-site movement and persistence of bensulide in soil. Eighteen plots of 22 × 3.7 m each were separated using metal borders and the soil in six plots was mixed with sewage sludge and yard waste compost (SS-YW) at 15 tons/acre on dry weight basis, six plots were mixed with sewage sludge (SS) at 15 tons/acre, and six unamended plots (NM) were used for comparison purposes. Plots were planted with summer squash, *Cucurbita pepo*, as the test plant. The objectives of this investigation were to: 1) determine the dissipation and half-life ($T_{1/2}$) of bensulide in soil under three management practices; 2) monitor the concentration of bensulide residues in runoff and infiltration water following natural rainfall; and 3) determine the effect of soil amendments on the transport of NO_3, NH_4, and P into surface and subsurface water. Half-life ($T_{1/2}$) values of bensulide in soil were 44.3, 37.6, and 27.1 days in SS-YW, SS, and NM treatments, respectively. Addition of SS-YW and SS to native soil increased water infiltration, lowering runoff water volume and bensulide residues in runoff following natural rainfall events.

Yield and Quality of Sweet Potatoes Grown in Municipal Sewage Sludge. ERIC T. TURLEY*, and GEORGE F. ANTONIOUS, Kentucky State University, Land Grant Program, Department of Plant and Soil Science, 210 Atwood Research Facility, Frankfort, KY 40601.

Demand for food is increasing and a lot of future plant production systems will depend on the use of fertilizers. Organic substances and nutrients in sewage sludge could be recycled and used for land farming. Field studies were conducted at Kentucky State University Research Farm, Franklin County, KY. Three soil management practices: 1) sewage sludge mixed with native soil at 15 tons/acre, 2) sewage sludge mixed with yard waste compost at 15 tons/acre, and 3) native soil, were used for growing sweet potato, *Ipomoea batatas* cv. Beauregard. Mature sweet potatoes were harvested and graded according to United States Department of Agriculture (USDA) Standards for Grades of Sweet Potatoes. Plants grown in sewage sludge-yard waste mix produced the greatest US No. 1 grade of sweet potatoes (smooth, well-shaped, and free from disease), US No. 2, total yield, and lowest weight of sweet potato culls compared to other two soil treatments. Total yield was increased by 53.7% in sewage sludge-yard waste treatments compared to the no- mulch soil. We concluded that sewage sludge mixed with yard waste compost might be a suitable technique to reduce dependence on synthetic fertilizers, restore and improve soil quality and crop yield.

Quantification of Napropamide and Trifluralin in Soil. RACHEL S. HAYDEN*, REGINA HILL, and GEORGE F. ANTONIOUS, Department of Plant and Soil Science, 218 Atwood Research Facility, Land Grant Program.TEJINDER S. KOCHHAR, Department of Biology, 219A Carver Hall, Kentucky State University, Frankfort, KY 40601.

The landfill crisis and other environmental concerns have resulted in a surge in recycling wastes as a natural means for using and detoxifying wastes and recovery of nutrients needed at all levels of life. Recycling sewage sludge for use as a soil amendment would reduce the need for landfill disposal and the impact of disposal on environmental quality. A field study was conducted at Kentucky State University Research Farm. Eighteen plots of 22 × 3.7 m each were separated using metal borders and the soil in six plots was mixed with sewage sludge, six plots were mixed with yard waste compost, and six unamended plots were used for comparison purposes. The soil was sprayed with trifluralin and napropamide herbicides. Runoff and infiltration water were collected following natural rainfall events and herbicide residues were quantified using GC/NP detector. The objectives of this investigation were to study: 1) the impact of soil amendments on the amount of runoff water down the land slope and infiltration water in the vadose zone, and 2) the impact of soil amendments on herbicide mobility under field conditions. Residues of trifluralin were significantly higher in sewage sludge treatments compared to yard waste and unamended soil. Napropamide residues in the vadose zone were 0.3 mg/acre in the NM treatment compared to 1.4 mg/acre in yard waste compost treatment. Addition of sewage sludge increased soil retention of trifluralin and napropamide residues, lowering their concentration in runoff, and reducing their transport into streams and rivers.

Heavy Metals in Squash Fruits Grown in Soil Amended with Sewage Sludge. GEORGE F. ANTONIOUS*, Department of Plant and Soil Science, 218 Atwood

Research Facility, Land Grant Program, Kentucky State University, Frankfort, KY 40601.JOHN C. SNYDER, Department of Horticulture, University of Kentucky, Lexington, KY 40546.SAM O. DENNIS, Department of Agricultural Sciences,Tennessee State University, Nashville, TN 37209.

The effects of amending soil with compost made from municipal sewage sludge (MSS) and MSS mixed with yard waste (MSS-YW) on Cd, Cr, Mo, Cu, Zn, Pb, and Ni concentrations in soil and the potential bioaccumulation of heavy metals in squash fruits at harvest were investigated. A field study was conducted in a silty-loam soil at Kentucky State University Research Farm. Eighteen plots of 22 × 3.7 m each were separated using metal borders and the soil in six plots was mixed with MSS at 15 tons/acre, six plots were mixed with MSS-YW at 15 tons/acre (on dry weight basis), and six unamended plots (no-mulch) were used for comparison purposes. Plots were planted with summer squash and heavy metals were analyzed in soil and mature fruits at harvest. Analysis of heavy metals in squash fruits was conducted using inductively coupled plasma spectrometry. Zinc and Cu concentrations in soil mixed with MSS were extremely high compared to other metals. Total squash marketable yield was greatest in MSS-YW and MSS treatments compared to no-mulch conventional soil. Concentrations of Cd and Pb in soil amended with MSS averaged 0.1 and 1.4 mg/kg, respectively. Maximum concentrations of Cd and Pb in squash fruits were 0.03 and 0.01 µg/g dry fruit, respectively. Nickel concentration in fruits fluctuated among harvest dates reaching a maximum of 2.5 µg/g dry fruit.

Soil Amendments Modified Antioxidant Content of Sweet Potato Roots. REGINA R. HILL*, and GEORGE F. ANTONIOUS, Kentucky State University, Land Grant Program, Department of Plant and Soil Science, 218-Atwood Research Facility, Frankfort, KY 40601.

A study was conducted at Kentucky State University Research Farm (Franklin County, KY) to determine the concentrations of ascorbic acid, phenol, β-carotene and soluble sugars in sweet potato (*Ipomoea batatas* L.) cv. Beauregard grown under three soil management practices. The soil management practices were municipal sewage sludge (SS), yard waste compost mixed with municipal sewage sludge (YW + SS), and no-mulch (NM) soil. Six replicates of each soil treatment were established in 18 plots of 22 × 3.7m each. No significant differences were found between ascorbic acid and phenols in sweet potato roots grown in YW + SS and SS but were both greater than concentrations in roots grown in NM treatments. Soluble sugars were greatest in YW + SS while β-carotene concentration was greatest in SS compared to YW-SS treatments (158 and 99 µg/g, respectively).

Analysis of Hot Pepper Fruits for Capsaicin Content. SHANAE KINCANNON*, and GEORGE F. ANTONIOUS, Department of Plant and Soil Science, Land Grant Program.TEJINDER S. KOCHHAR, Department of Biology, Carver Hall, Kentucky State University, Frankfort, KY 40601.

Capsaicin is the most pungent of the group of compounds called capsaicinoids in chili peppers. A survey was conducted to screen 90 hot pepper accessions selected from the USDA germplasm collection for their major capsaicinoids content. Fresh fruits of *Capsicum chinense*, *C. frutescens*, *C. baccatum*, *C. annuum*, and *C. pubescens* were extracted with methanol, and analyzed for capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide), dihydrocapsaicin (8-methyl-N-vanillylnonanamide), and nordihydrocapsaicin. Mass spectrometry of the fruit crude extracts indicated that the molecular ions at m/z 305, m/z 307, and m/z 293, which correspond to capsaicin, dihydrocapsaicin, and nordihydrocapsaicin, respectively, have a common benzyl cation fragment at m/z 137 that can be used for monitoring capsaicinoids in hot pepper extracts. Capsaicin and dihydrocapsaicin were the dominant capsaicinoids detected in *Capsicum* species. Capsaicin was higher than dihydrocapsaicin and total content of capsaicinoids varied between species and accessions of the same species from none detectable to 11.2 mg/g fresh fruit. Accession PI-441624 (*C. chinense*) had the greatest capsaicin content (2.9 mg/g fresh fruit) and accession PI-497984 (*C. frutescens*) had the greatest dihydrocapsaicin content (2.3 mg/g fresh fruit), while PI-439522 and PI-497984 (*C. frutescens*) are the two accessions containing the highest concentration of total capsaicinoids.

Effect of Different Schedules of Baby Corn (*Zea mays* L.) Harvests on Baby Corn Yield, Grain Yield, and Economic Profit Value. ZHENG WANG*, ELMER GRAY, and MARTIN STONE, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

Maize (*Zea mays* L.) ranks third as a food crop after wheat and rice and is characterized not only as a cereal crop but also as a vegetable. Maize used as a vegetable is known as "baby corn." Baby corn consists of unfertilized young ears harvested two or three days after silk emergence. The present study was implemented in 2009 at Western Kentucky University Agriculture Research and Education Center (36.93 N, 86.47 E) in Bowling Green, Kentucky. The purpose of the study was to compare the effect of different schemes of harvest on baby corn (BC) yield, grain maize (GM) yield, and estimated economic return. Experimental harvest treatments were 1) no BC harvest, only GM harvest, 2) first harvest as BC, final harvest as GM, 3) first and second harvests as BC, final harvest as GM, and 4) first, second, and third harvests as BC, final harvest as GM. Average estimated BC yields (kg/ha) for treatments 2, 3, and 4 were 1445.1, 2681.8, and 3437.5; GM yields (kg/ha) for treatments 1, 2, and 3 were 12522.2, 8226.5, and 1380.9; respectively. Since few grain kernels were found after three harvests for BC (harvest 4), no usable GM yield was produced. BC and GM yields were used for evaluating the economic returns. Results

indicated that the sequence of best economic returns would be obtained by harvesting BC three times (treatment 4), first two harvests for BC and the final for GM (treatment 3), first harvest for BC and subsequent for GM (treatment 2), and only for GM harvest (treatment 1). Although the pattern for only BC harvest was the most profitable system, the human labor requirement and critical timing of harvest limited its production. In states similar to Kentucky, BC could only be grown as an additional crop or to supplant a limited amount of traditional GM hectarage.

Flower and Synthetic HIPPO Attractancy of Four Common Lady Beetle Species. JOHN D. SEDLACEK[1]*, DANIEL J. SEDLACEK[2], and KAREN L. FRILEY[1]. [1]Community Research Service, Kentucky State University, Frankfort, KY 40601, and [2]St. Leo School, Versailles, KY 40383.

Lady beetles have been reported to be attracted to flowering plants, including species in the aster family such as goldenrods, *Solidago* spp.; button mums, *Chrysanthemum* spp.; daisies, *Chrysanthemum* spp.; and sunflowers, *Helianthus* spp. It has also been reported that odors given off by crop plants as they are wounded or being fed upon by some types of insects also attract beneficial insects. These odors are known as HIPPOs, or herbivore induced plant protection odors. Laboratory experiments using an olfactometer were conducted to determine the attractiveness of goldenrod, button mum, daisy, sunflower, two commercially available beneficial insect lures containing synthetic herbivore plant protection odors, and their active ingredients for the convergent lady beetle, *Hippodamia convergens*; multicolored Asian lady beetle, *Harmonia axyridis*; pink lady beetle, *Coleomegilla maculata*; and the seven-spotted lady beetle, *Coccinella septempunctata*. All experiments were conducted at room temperature. Responses of the lady beetles to the flowers and synthetic volatiles varied from 10 to 90%. Pink lady beetles were attracted by goldenrod and daisy, but repelled by button mum. Multicolored Asian lady beetles were also repelled by button mum. Pink lady beetles were attracted by methyl salicylate-based PredaLure® and oil of wintergreen. The 2-phenylethanol-based Benallure® repelled pink and multicolored Asian lady beetles. Reports of lady beetle attractancy to various flowering plants and synthetic lures appear to be too general or inaccurate. Results will be discussed with respect to habitat manipulation for conservation biological control.

Beneficial Insects in Organically Grown Late-Planted Sweet Corn Baited with Methyl Salicylate-Based PredaLure® Insect Attractant. LESLYE S. BRENT*, JOHN D. SEDLACEK, KAREN L. FRILEY, and MICHAEL K. BOMFORD, Community Research Service, Kentucky State University, Frankfort, KY 40601.

Sweet corn, *Zea mays* 'Garrison', was grown in 260 m^2 replicated plots using organic production practices. Plots were baited with PredaLure® or were left as non-baited controls. One lure was fastened to a tobacco stick placed in the center of the plot and in the center of each plot quadrant at crop canopy height. Beneficial insects were sampled weekly during silking using 232 cm^2 yellow sticky traps stapled to each tobacco stick at ear height. Pink lady beetles, *Coleomegilla maculata*; Asian lady beetles, *Harmonia axyridis*; spotless lady beetle, *Cycloneda munda*; seven-spotted lady beetle, *Coccinella septempunctata*; parenthesis lady beetle, *Hippodamia parenthesis*; convergent lady beetle, *Hippodamia convergens*; big eyed bug, *Geocoris punctipes*, and green lacewing, *Chrysoperla carnea*, were the predatory insects collected on the traps. During early crop tasseling, Asian lady beetle was the most abundant predator caught followed by the big eyed bug and the pink lady beetle. All other predators were rare. There was a tendency toward higher numbers of multicolored Asian lady beetles and pink lady beetles in plots where PredaLure lures had been deployed. However, there were no significant differences in abundance of any of the predatory insects found between PredaLure baited and non-baited plots in organically grown sweet corn. This could be due to large numbers of weeds present (e.g., pigweed, *Amaranthus hybridus*) in baited and non-baited plots obscuring methyl salicylate plumes. Other possible explanations may involve the rate of emission of the methyl salicylate from lure dispensers or placement of the lures in the crop.

Can Papwpaw Fruit Extract Be Used to Manage Striped Cucumber Beetle on Squash? KAREN L. FRILEY*, JOHN D. SEDLACEK, JEREMIAH D. LOWE, and KIRK W. POMPER, Community Research Service, Land Grant Program, Kentucky State University, Frankfort, KY 40601.

Laboratory experiments were performed to determine the effects of pawpaw (*Asimina triloba*) fruit extract on mortality and feeding deterrence of striped cucumber beetle (*Acalymma vittatum*). Recently, fruit tissues of pawpaw were found to contain annonaceous acetogenin compounds having insecticidal activity. Ripe pawpaw fruit pulp was extracted with 100% ETOH to obtain acetogenin compounds. Pulp extracts of 0, 10, 100, 1,000, 10,000 and 50,000 ppm were used to assess feeding deterrence and mortality of beetles. Buttercup squash leaf disks 3.5 cm in diameter were treated individually with each concentration and placed on water moistened filter paper in 9 cm plastic Petri dishes. Five striped cucumber beetles were placed on each leaf disk. All Petri dishes were then placed in an environmental growth chamber set at 27°C and a 16: 8 hr light: dark photo period. After 24 hr the cucumber beetles were removed. An LC_{50} of 50,538 ppm was calculated for the extract concentration required to kill 50% of the beetles. Pawpaw fruit extract reduced feeding by 90% and 98% in the 10,000 and 50,000 ppm treatments, respectively. At 10,000 ppm 10% of the beetles were killed, however only 10% of the leaf tissue was consumed. A LFC_{10} value of 2,033 ppm was calculated for the extract concentration required to limit

feeding to 10% of the leaf. This suggests that pawpaw fruit extracts may be effective insect feeding deterrents. The duration of treatment effectiveness and susceptibility of other pest and beneficial insect species to the extracts should be examined.

The Economic Performance of Mulch Color and Cultivar Selection for Tomato (*Lycopersicon esculentum*) Production in Kentucky. STEPHEN A. KING*, MARTIN J. STONE, and STEPHEN T. FLOMO, Department of Agriculture, Western Kentucky University, 1906 College Heights Blvd., #41066, Bowling Green, KY 42101.

Farmers seeking to earn a profit are interested in selecting products and production methods that will earn them the highest return possible. Among certain segments of the population there is a growing interest in locally grown fresh produce; this interest is revealed in Kentucky by numerous phenomena such as the consumers who patronize and support our farmers' markets as well as the "Kentucky Proud" program. These opportunities for farmers may be only partially realized due to a lack of research on specific crop-production system combinations, specifically high value-added crop-production systems such as heirloom tomatoes and plastic mulch colors. In order to aid producers of tomatoes, Western Kentucky University established a research program focusing on the production of tomatoes. Our research focused on preliminary results of a partial budgeting analysis of the profitability of various combinations of mulch color and tomato cultivars. Yields, revenues, costs, and profits of six cultivar-production system combinations were estimated. These combinations were derived from two colored mulches (red and black) that were employed in the production of three cultivars of tomatoes, 'Cherokee Purple' and 'Mr. Stripey' which are heirlooms, and 'Crista' a commercial standard. The results of the research indicated that Mister Stripey produced with red plastic mulch delivered the highest per acre profit, followed in descending order by Mister Stripey on black, Cherokee Purple on black, Crista on black, Cherokee Purple on red, and lastly Crista on red.

Evaluation of New Simple Sequence Repeat Markers for Pawpaw (*Asimina triloba*) Genetic Diversity Analysis. CHANIECE DAVIES*, LI LU, KIRK W. POMPER, JEREMIAH D. LOWE, SHERI B. CRABTREE, and LAUREN A. COLLINS, Land Grant Program, Kentucky State University, Atwood Research Facility, Frankfort, KY 40601-2355.

Pawpaw [*Asimina triloba* (L.) Dunal] is a tree-fruit native to the southeastern region of the United States. The tree can grow up to 12 meters in height, and bear nutritious fruits which are rich in amino acids. Kentucky State University serves as the USDA National Clonal Germplasm Repository for pawpaw. Research concerning pawpaw genetic diversity and DNA fingerprinting are priorities, and an efficient DNA marker system is essential for conducting the research. The objective of this study was to test if newly developed Simple Sequence Repeat (SSR) DNA markers are polymorphic or monomorphic. The SSR markers were developed based on pawpaw genomic libraries for the di-nucleotide repeat GA, and for tri-nucleotide repeats ATG and AAT. After primary selection with agarose gel electrophoresis system, thirty-three pairs of SSR markers were selected and will be used for further Polymerase Chain Reaction (PCR) with DNA extracted from different pawpaw cultivars. Twenty SSR primers were labeled with FAM or HEX for further study and used to amplify ten pawpaw varieties. The SSR-PCR products will be separated using a 3130 Applied Biosystems capillary electrophoresis system. The observed number of alleles at each locus, sizes of the alleles, number of genotypes, and allele scoring quality of the markers will be reported.

Using Simple Sequence Repeat Markers to Distinguish Scion and Rootstock Tissues on Grafted Pawpaw (*Asimina triloba*) Trees. MYESHA HOLLINS*, LI LU, KIRK W. POMPER, JEREMIAH D. LOWE, and SHERI B. CRABTREE, Land Grant Program, Kentucky State University, Atwood Research Facility, Frankfort, KY 40601-2355.

The pawpaw [*Asimina triloba* (L.) Dunal] is a tree-fruit that is native to the southeastern region of the United States. Kentucky State University serves as the USDA National Clonal Germplasm Repository for pawpaw; therefore, research concerning pawpaw genetic diversity and DNA fingerprinting are priorities. Pawpaw varieties are propagated by placing a bud, or scion, from a selected variety onto a seedling root system. Scion death and the formation of shoots from the root system result in a tree which is no longer true to the original variety. Usually trees that develop from rootstock shoots produce poor quality fruit that is inferior to the scion variety. The objective of this study was to determine if scions of trees ('Sunflower' or 'Susquehanna') in the KSU Scion/rootstock trial are genetically true to type or have been replaced by shoots from the rootstock. Leaf samples were collected from varieties 'Sunflower', 'Susquehanna', and seven trees that may not be true to type in the KSU pawpaw rootstock trial. Additional leaf samples of wild pawpaw trees in Cove Spring Park in Frankfort, KY were used as a secondary control. DNA was extracted from the leaves using the DNAMITE Plant Kit. Primers B3, B103, B129, and G119 were used to amplify SSR products. These products were then separated using a 3130 Applied Biosystems capillary electrophoresis system. Trees in the Cove Spring patch showed a range of pawpaw genotypes. Scions of six of the seven trees were not true to type and were rootstock derived trees that should be removed.

Seedling Rootstock, Scion Cultivar, and Pruning Effects on Yield of Pawpaw. SHERI B. CRABTREE*, KIRK W. POMPER, and JEREMIAH D. LOWE, Land Grant Program, Atwood Research Facility, Kentucky State University, Frankfort, KY 40601.

The pawpaw [*Asimina triloba* (L.) Dunal] is a tree fruit indigenous to the eastern United States. Pawpaw is increasing in interest as an alternative niche crop. Rootstocks for pawpaw cannot be propagated clonally; therefore, nurseries graft cultivars onto seedling rootstock grown from genetically diverse seed, which could impart a high rate of variation in vigor, precocity, and yield to the resulting grafted tree. Pawpaw rootstocks that are vigorous, have a high survival rate, and promote precocity and high yields would be desirable to growers. Alternative pruning methods have also not been examined in pawpaw. A field planted rootstock trial was conducted to determine if survival, vigor, precocity, and yield of scions would vary by seedling rootstock source, scion, and pruning method (central leader vs. minimal). Most seedling rootstocks showed a high survival rate during field establishment (71% survival across all rootstocks); however, rootstock derived from seeds of 'Susquehanna' showed a higher mortality rate (52% survival) and therefore is not recommended for use as pawpaw rootstock. Vigor was only affected by pruning method, with minimally pruned trees having a larger trunk cross-sectional area. 'Sunflower' scions were the most floriferous, while scions grafted onto 'K8-2' rootstock produced the fewest flowers. 'Susquehanna' pawpaw fruit had an average fruit weight of 258 g, while 'Sunflower' fruit averaged 190 g. Rootstock also had an effect on fruit size, with fruit produced by scions grafted onto 'K8-2' rootstock having a lower fruit weight than those grafted onto RVT, 'Sunflower', and 'NC-1' seedlings, which produced larger fruit.

Identification of the Optimal Solvent for Acetogenin Compound Extraction from Pawpaw Ripe Fruit Pulp. DOROTHY BADOO*, JEREMIAH D. LOWE, KIRK W. POMPER, SHERI B. CRABTREE, HIDEKA KOBAYASHI, and CHANGZHENG WANG, Land Grant Program, Kentucky State University, Atwood Research Facility, Frankfort, KY 40601-2355.

Pawpaw [*Asimina triloba* (L.) Dunal] is a tree fruit native to eastern North America. This plant contains annonaceous acetogenins in the twigs, fruit, seeds, roots, and bark tissues which display antitumor, pesticidal, antiviral, and antimicrobial effects, suggesting many potentially useful applications. The objective of the experiment was to identify the optimal solvent for acetogenin compound extraction from pawpaw ripe fruit pulp. About 10 grams of thawed fruit pulp from the cultivar 'Susquehanna' was extracted with acetone, ethanol, isopropanol, or methanol. The brine shrimp test (BST) was employed to assess acetogenin activity. Concentrated extract was transferred to vials to correspond to 0, 1, 5, 10, and 100 ppm concentrations. Ten brine shrimp larvae, taken 48 h after initiation of hatching in artificial sea water, were added to each vial, and the final volume of each vial was adjusted to 5 ml using the artificial sea water. After 24 h, survivors were counted. Four replicate extractions were preformed and three subreplicates were used for each extraction to conduct the BST assay. Acetone, ethanol, isopropanol, and methanol all yield similar extract weights. Acetone, ethanol, and isopropanol had similar extract activity in the BST as indicated by brine shrimp mortality rates and the LC_{50} value for each solvent; however, methanol extracts displayed lower (50%) biological activity levels compared to the other three solvents. Acetone, ethanol, and isopropanol appear to be excellent solvents for the extraction of acetogenin compounds, and possibly other bioactive compounds, from pawpaw fruit pulp. Screening for high acetogenin pawpaw cultivars has begun.

Third Year Yield Characteristics of Advanced Floricane Fruiting Blackberry Selections from the University of Arkansas Grown in Kentucky. JEREMIAH D. LOWE, KIRK W. POMPER, and SHERI B. CRABTREE, Department of Plant and Soil Science, Kentucky State University.JOHN R. CLARK, Department of Horticulture, University of Arkansas.JOHN G. STRANG, Department of Horticulture, University of Kentucky.

Blackberries offer an attractive alternative to Kentucky farmers who are currently growing tobacco. The objective of this study was to compare production characteristics from a number of advanced floricane fruiting selections, including Natchez, developed by the University of Arkansas Blackberry Breeding Program, to the commonly grown selections Chickasaw and Triple Crown in terms of yield and fruit quality under Kentucky growing conditions. In June 2006, a blackberry variety trial was established at Kentucky State University (KSU). The variety trial includes the commercially available cultivars Chickasaw (thorny erect) and Triple Crown (semi-erect, thornless) and the Arkansas (A) floricane fruiting selections A-1937T, A-2215T, A-2241T, and A-2315T; the selection A-2241T was released in 2008 as Natchez after the start of the trial. All the advanced selections are thornless and erect in stature. The advanced selections and two commercially available cultivars were planted at the KSU Research and Demonstration Farm in Frankfort, KY. Plants were arranged in a completely randomized design; with two replicate plots each containing five plants of each selection or cultivar (total of ten plants of each selection or cultivar) in a ten foot plot. This trial was managed with organic practices following the National Organic Program standards. Ripe fruit were harvested from the plants twice weekly from July until August. Harvest period, yield, and berry weight were recorded for all selections. Chickasaw had the highest yield followed by Natchez. Apache had the largest berry followed by Natchez. Natchez appears to be a desirable cultivar well suited to Kentucky growing conditions.

BOTANY

Vascular Flora and Plant Habitats of the Camp Nelson Abandoned Limestone Quarry in Garrard County, Kentucky. RALPH L. THOMPSON*, Berea College Herbarium, Department of Biology, Berea College, Berea, KY 40404.STEPHANIE R. GREEN, Southwest

Florida Water Management District, 170 Century Boulevard, Bartow, FL 33830.

A floristic survey of the vascular plants from nine plant habitats was conducted on the Camp Nelson Quarry, a limestone quarry abandoned since 1991, in Garrard County, Kentucky. Thirteen collecting trips were made during 1996, 1997, 2000, 2003, and 2004 with all voucher specimens deposited in the Berea College Herbarium. The annotated species list consisted of 209 species in 155 genera from 61 families. The known vascular plants included one Pteridophyta, one Gymnosperm, and 207 Angiosperms (45 Monocots, 162 Dicots). A total of 137 taxa (65.6%) were native and 72 (34.4%) were exotic. Thirty-four exotics are considered invasive pest plants for Kentucky. Thirty-four taxa (16.3%) were woody and 175 (83.7%) were herbaceous annuals, biennials, and perennials. During 13 years of disuse, plants have colonized nine anthropogenic habitats in the quarry through progressive secondary succession. These habitats included vertical highwalls, talus slopes, spoil heaps, dry quarry floor, wet quarry floor, old-field succession areas, wet ditch border, seasonal pond area, and permanent deep pond area. Limestone quarry environments are major examples where progressive secondary succession occurs under harsh circumstances and relatively high species richness exists. Sørenson's Index of Similarity calculated between the Camp Nelson Quarry and a 39 year-old abandoned quarry in Clark County was 57.6%. A higher similarity coefficient will result as time and progressive secondary succession continues.

Evaluation of Genetic Variation Among Native Pawpaw Patches at the Environmental Education Center at Kentucky State University. LAQUIDA BOWIE*, LI LU, KIRK W. POMPER, JEREMIAH D. LOWE, SHERI B. CRABTREE, and WILLIAM STILWELL, Land Grant Program, Kentucky State University, Atwood Research Facility, Frankfort, KY 40601-2355.

Pawpaw [*Asimina triloba* (L.) Dunal] is a tree-fruit that is a native understory tree in the eastern region of the United States. How pawpaw patches have spread in this region is poorly understood. Native Americans may have spread seed to promote fruit production. Animals may consume fruit and spread seed in scat. Viable pawpaw seeds are buoyant and can float down streams to form new patches. Clonal patches can develop through root suckering. The objective of this study was to determine if DNA fingerprinting of patches at the KSU Environmental Education Center in Henry County, Kentucky near a stream would suggest if root suckering (clonal patches) or water transport of seeds (many genotypes) were mechanisms of patch establishment. DNA was extracted using the DNAMITE Plant Kit from leaf samples collected from 20 trees per patch on a transect line in three native patches at the EEC. Patch 1 was located on a hill, while patches 2 (downstream) and 3 (upstream) were located on a stream about 100 feet apart. Primers B3, B103, B129, C104, and G119 were used to amplify SSR products, and products were separated with a 3130 Applied Biosystems capillary electrophoresis system. All three patches were genetically distinct, with patches 2 and 3 being more similar. Patch 3 was clonal with only one genotype. The high genetic variation in patch 2 supports involvement of water transport of seed from patch 3 in the establishment of the patch; however, animal transport of seed could also have led to patch establishment.

ECOLOGY AND ENVIRONMENTAL SCIENCE

Effects of Hydrologic Gradients on Woody Debris Breakdown and Macroinvertebrate Colonization Patterns in a Cumberland Plateau Watershed in Eastern Kentucky, U.S.A. ROBIN R. BERNAL* and SCOTT A. GRUBBS, Department of Biology and Center for Biodiversity Studies,1906 College Heights Blvd. #11080, Western Kentucky University Bowling Green, KY 42101.

This research assessed the influence of hydrologic gradients on woody debris dynamics in a Cumberland Plateau watershed, eastern Kentucky, U.S.A. Although the breakdown of wood can be attributed to several different processes, including leaching, fragmentation, transport, respiration, and biological decay, the influence of differing flow regimes has been unstudied. The objectives of this study were to examine how stream channel type (temporary vs. perennial) affects wood processing dynamics (i.e., mass loss, macroinvertebrate biomass, and macroinvertebrate colonization patterns). Two questions were addressed: (1) do mass loss rates of wood differ across hydrological gradients in stream channels? and (2) do macroinvertebrate colonization patterns vary in relation to hydrologic gradients? Wood bundles of 10 g of red maple heartwood strips were placed in nylon mesh bags and placed in temporary and perennial channels of eight streams in January 2008. Three replicate bundles were retrieved from each ephemeral and perennial channel on a monthly basis. Analysis of dry mass loss over a 6-month in-stream incubation period showed that there was no significant difference ($p < 0.05$) within either channel type. Similarly, a comparison between channel types revealed no significant influence ($p < 0.05$) of hydrologic permanency of dry mass loss. Although macroinvertebrate analyses are nearly complete, the similarities in woody debris mass loss rates between intermittent and perennial channels suggest that hydrologic gradients were relatively shallow.

Restoration of Native Warm Season Grassland in a Kentucky Tall Fescue Pasture Using Prescribed Fire and Herbicides. SARAH L. HALL[1]*, ROBERT J. BARNEY[1] and REBECCA L. MCCULLEY[2]. [1]Kentucky State University, Community Research Service, Frankfort, KY 40601, and [2]University of Kentucky, Department of Plant and Soil Sciences, Lexington, KY 40546-0091.

Land managers have had varying degrees of success eradicating tall fescue using herbicides and prescribed burns to restore native warm season grasslands. A

mutualistic symbiosis between tall fescue and a fungal endophyte may increase the plant's ability to withstand herbicide and fire and account for some of the variability in restoration success across the state. In fall 2008, we assessed restoration success at Crooked Creek Barrens State Nature Preserve within one field divided into five sub-units, each having different numbers of herbicide applications and prescribed burns between 1999 and 2004, including a control with no management. Our objectives were: 1) to assess if restoration practices decreased tall fescue and increased species diversity; and 2) to evaluate whether restoration efforts select for endophyte-infected tall fescue plants. Tall fescue cover was lower in all units than levels prior to restoration, but other exotic species increased following restoration. Although species richness was highest in the control unit, the species composition indicated succession to forest was occurring in this area whereas native grassland species dominated the restorations. Fungal endophyte levels did not increase following restoration and were remarkably low in all sub-units. We hypothesize that these low infection rates might be responsible for the overall success at reducing tall fescue at this site and the rapid succession of the control unit. Surveys across restoration sites throughout the state where tall fescue eradication has been more difficult indicate that the low endophyte infection frequency encountered at this site is unusual.

GEOGRAPHY

Hidden Land Cover: Assessing Dynamic Change in Small Water Bodies with Landsat 5 and Aerial Images and Derivatives. DEMETRIO P. ZOURARAKIS*, Kentucky Division of Geographic Information, Frankfort, KY 40601.

High intensity agricultural, forestry, urbanization and mining activities in Kentucky determine dynamic changes in the total number and locations of ponds and reservoirs, critical in providing fresh water resources for a wide diversity of uses to livestock, crops, industry, commerce and humans. Inventorying these water bodies is often challenging due to the patterns of land use and ownership in the Commonwealth. The wetness band of tasseled-cap-transformed, 2005 epoch (leaf-on) Landsat 5 Thematic Mapper data and high resolution aerial imagery (leaf-on, 2004, 2006 and 2008) were used to detect and photo-verify both pre-existing, and new but unmapped bodies of water in Martin County, Kentucky. The "known" water bodies were extracted from the National Hydrography Dataset, and used as a mask to calculate pixel-based statistical measures for the wetness values: 109.7 ± 10.4 (average and standard deviation), 108.7 (median), and 106.3 (majority). By using a threshold wetness band pixel values equal to 134 as an indicator of open water, several previously unmapped water bodies were discovered. This method, however, failed to detect smaller features possibly due to the low spatial resolution, and also yielded some false positives.

HEALTH SCIENCES

Preliminary Investigation into Human Life Expectancy as Influenced by Country, Age Scale, and Gender for the Period of 1990 to 2006. PRAMOD R. GUPTA*, ZHENG WANG, and ELMER GRAY, Departments of Public Health and Agriculture, Western Kentucky University, Bowling Green, KY 42101.

Life expectancy (LE) is critical to both the individual person as an indication of mortality and to governments and other public agencies that provide support based upon age. Length of human life is a major factor in population planning. LE for different age groups reflects the status of public health service. The World Health Organization (WHO) reported LE information by country, gender, and age scale. Using WHO data for 2006, it was determined that 40% of the countries had LE at birth between 70 and 79 years; approximately 77% had LE of 60+ years. Changes in LE were analyzed for the period between 1990 and 2006. Results indicated that LE for this period increased for the 190 countries overall; however, statistically, 73% increased, 20% decreased, and 7% were unchanged. For the 190 countries, changes in LE for both genders were greater at the <1-year scale and decreased progressively through the 100+ scale. This decrease followed similar patterns for males and females resulting in a significant linear correlation ($r = 0.879$). The change for males exceeded that of females for birth to about 40 years while change in females exceeded that of males in subsequent years. This interaction between genders and age scales resulted in approximately the same average increase (1 year) for males and females. These results indicated that LE increased overall but not uniformly over countries, that the rate of increase may be slightly higher in males than females, and that increases are greater for younger than older ages. The increase in LE will add to the human population and to the need for public health service.

PSYCHOLOGY

The Impact of Studying AD/HD Diagnostic Criteria and Case Histories on Successful Malingering on the Adult AD/HD Self-Report Scale-v.1.1. KATHERIN AUSTIN*, AMY KISER, LEAH SMITH, and SEAN P. REILLEY, Department of Psychology, Morehead State University, Morehead, KY 40351.

Attention Deficit/Hyperactivity Disorder (AD/HD) is a frequently diagnosed psychological disorder in adults and is characterized by hyperactive-impulsive and/or inattention symptoms. Behavioral rating scales like the Adult AD/HD Self-Report Scale-v.1.1 (ASRS-v.1.1) are assessment tools that are used to screen individuals for current symptoms of AD/HD. Limited information exists concerning the impact of studying freely available AD/HD information, such as diagnostic criteria, on successful malingering on the ASRS-v.1.1. Because the ASRS-v.1.1 has no items to assess malingering and similar scales are substantially compromised by malingering, the ASRS-

v.1.1 was expected to be negatively impacted by AD/HD malingering. Using an experimental approach, the current study (N = 30) tested and found that studying AD/HD diagnostic and case history information led to increased knowledge about AD/HD and clinically elevated ASRS–v.1.1 scores. The findings are discussed for using the ASRS-v1.1 for adult ADHD assessment. Research supported by a prior grant from KY EPSCoR.

The Ability of the CAT-A to Detect Malingered Current AD/HD Symptoms in Adults With and Without Enhanced AD/HD Knowledge. MEDINA JACKSON*, and SEAN P. REILLEY, Department of Psychology, Morehead State University, Morehead, KY 40351.

Attention Deficit/Hyperactivity Disorder (AD/HD) is a life span disorder with significant impact on daily living due to hyperactivity, impulsivity, and/or inattentive symptoms. Malingered AD/HD in adults is an area of concern given varying levels of knowledge and assessments among providers and high potential for secondary gain among clients. Although a number of adult-oriented attention rating scales provide self-reported information concerning the presence of current AD/HD symptoms, only the Clinical Assessment of Attention Deficit-Adult (CAT-A) assesses the validity of symptom reporting. Using an experimental approach, the current study (N = 65) tested and found that studying either diagnostic symptoms or a case history of an adult with AD/HD was sufficient to enhance knowledge of AD/HD. In addition, the CAT-A more accurately detected malingering of current AD/HD symptoms in adults compared to another popular AD/HD measure in both case history and diagnostic symptom information conditions. The results are discussed in relation to adult AD/HD assessment. Research supported by a Morehead State University Undergraduate Research Fellowship and a prior grant from KY EPSCoR.

The Reliability and Validity of the Adult Knowledge of Attention Deficit Disorders Scales. JULIA DAHMANE*, and SEAN P. REILLEY, Department of Psychology, Morehead State University, Morehead, KY 40351.

Attention Deficit/Hyperactivity Disorder (AD/HD) is a lifespan disorder that affects all age groups. Currently there is no published instrument for determining an adult's knowledge of AD/HD symptoms, associated features, and successful treatments. Having an adult AD/HD knowledge instrument could be helpful for measuring the impact of psychoeducational interventions for clients, teachers, and professionals. The Adult Knowledge of Attention Deficit Disorders Scales (AKADDS) was modeled on a published child AD/HD knowledge scale and refined using findings in the empirical adult AD/HD literature to measure adults' knowledge of diagnostic symptoms, treatment factors, and associated features of AD/HD. The present study (N = 117) replicates and extends pilot work associated with this instrument. Specifically, the AKADDS was hypothesized and found to have acceptable internal consistency, good convergent validity with a child AD/HD knowledge measure, and displayed increases in knowledge scores associated with studying AD/HD information. The findings are discussed in relation to AD/HD assessment and treatment. Research supported by a prior grant from KY EPSCoR.

Preliminary Evaluation of the Detection of Malingered Childhood AD/HD Symptoms in Adults on the CAT-A. KELLY D. GRUBER*, NORA WEYH, and SEAN P. REILLEY, Department of Psychology, Morehead State University, Morehead, KY 40351.

Attention Deficit/Hyperactivity Disorder (AD/HD) is a disorder characterized by symptoms of hyperactivity/impulsivity, inattention, or both symptom types. Due to the increasing interest in AD/HD among adults, concerns about malingering the disorder for secondary gain have also increased. This is especially true for past reporting of childhood AD/HD symptoms, an assessment area this more difficult to corroborate in adults. Few behavioral rating scales exist that can adequately evaluate the validity of self-reported attention problems. A new instrument, the Clinical Assessment of Attention Deficit-Adult (CAT-A), includes three validity scales; two of these scales were the focus of our study: negative impression and symptom infrequency. Using an experimental approach, the current study (N = 40) tested and found that the validity subscales of the CAT-A allowed the instrument to significantly outperform the childhood symptoms scale of the Barley and Murphy instrument in accurately detecting faked adult AD/HD in college adults with no history of AD/HD. The results are discussed in relation to adult AD/HD assessment. Research supported by a Morehead State University Undergraduate Research Fellowship and a prior grant from KY EPSCoR.

The Impact of Reviewing Information from Multiple AD/HD Case Studies on AD/HD Knowledge and Malingering of AD/HD Symptoms on the Barkley & Murphy AD/HD Scales. TINA WARD*, MEDINA JACKSON, JONATHAN RAKES, and SEAN P. REILLEY, Department of Psychology, Morehead State University, Morehead, KY 40351.

Attention Deficit/Hyperactivity Disorder or AD/HD is a frequently diagnosed mental health disorder in children, adolescents, and adults. Attention rating scales such as the Barkley & Murphy AD/HD Scales, are among the most commonly used measures for screening and evaluating AD/HD in adults. Limited research has addressed the impact of studying more than one source of AD/HD information on AD/HD knowledge and subsequent maligning of adult AD/HD. Using an experimental approach (N = 34), the current study tested whether studying one or three published psychiatric case studies about adult patients with AD/HD would lead to increased AD/HD knowledge, and successful AD/HD malingering on the Barkley & Murphy AD/HD scales. Our data suggests studying detailed case histories of adults with

AD/HD was associated with significantly enhanced AD/HD knowledge relative to controls and success in malingering both childhood and current AD/HD symptoms on the Barkley & Murphy Scales. The results are discussed in relation to adult AD/HD assessment. Research supported by a prior grant from KY EPSCoR.

PHYSIOLOGY AND BIOCHEMISTRY

Energy Costs and Trade-offs of the Adaptive Immune System in Old-field Mice (*Peromyscus polionotus*). SARAH HARGIS*, RENEE LEVESQUE, and TERRY L. DERTING, Department of Biological Sciences, Murray State University, Murray, KY 42071.

A high energetic cost of adaptive immune defenses is assumed in theoretical discussions of immune responses in animals. Little quantitative data is available to test the assumption, however, especially for mammalian species. We tested the null hypotheses that 1) there is no difference between energy expenditure of challenged and static cell-mediated and humoral immune systems, and 2) there is no change in the cost and magnitude of a humoral immune response when a cell-mediated immune response is introduced. To test these hypotheses, we used a two-by-two experimental design with humoral (sheep red blood cells) and cell-mediated (2,4-dinitrofluorobenzene) challenges as the independent variables. Using adult male old-field mice (*Peromyscus polionotus*), we measured hemagglutination, inflammation, metabolic rates, and organ masses to assess the energetic cost and potential energy trade-offs associated with cell-mediated and humoral immunity, and interactions between them. We rejected our first hypothesis. The cell-mediated, but not the humoral immune response, was associated with a 13.7% increase in resting metabolic rate (RMR), while neither response was associated with a significant change in daily metabolic rate (DMR). Also, the cell-mediated response was correlated with a significant decrease in testes mass and colon length. Reduced organ sizes may indicate that part of the cost of mounting a cell-mediated response was met through reduced energy allocation to the digestive and reproductive systems. The cell-mediated response had no measureable effect on the humoral response. Our results supported the assumption of a significant energetic cost of cell-mediated, but not humoral immune defense.

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INDEX TO VOLUME 70

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CONTENTS